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CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 4 APRIL, 1944 NUMBER 4

# The Spectrographic Analysis of Carcinogenic Hydrocarbons and Metabolites

## IV. The Elimination of 1,2,5,6-Dibenzanthracene from the Rat*

R. Norman Jones, Ph.D., C. E. Dunlap, M.D., and C. J. Gogek, B.Sc.

*(From The Chemistry Department, Queen's University, Kingston, Canada; The Converse Memorial Laboratory, Harvard University, Cambridge, Massachusetts; and The Department of Pathology, Harvard Medical School, Boston, Massachusetts)*

(Received for publication November 29, 1943)

### INTRODUCTION

A great number of substances have been synthesized that are capable of inducing malignant tumors in experimental animals, but only during the last few years has information become available concerning the metabolism of some of these compounds. The elimination of 1,2,5,6-dibenzanthracene [1] and 3,4-benzpyrene [1] has been studied more completely than that of other carcinogens of the aromatic hydrocarbon type.

When dibenzanthracene is injected into rats or mice (15, 16) it is metabolized in part to a dihydric phenol, which Cason and Fieser (10) have characterized as 4′,8′-dihydroxydibenzanthracene. The rabbit converts the same hydrocarbon, administered orally (8) or injected subcutaneously (15, 16), into an isomeric dihydroxydibenzanthracene, the structure of which has not yet been established.

Chalmers and his co-workers (11—14) showed that when benzpyrene is injected subcutaneously into the rat it is metabolized to a fluorescent, alkali-soluble derivative that can be readily observed in the bile and feces, but is present in traces only in the urine. This metabolite, which was designated as BPX by Peacock (24), is accompanied by lesser amounts of benzpyrene. Berenblum and Schoental (1, 2) increased the output of BPX by injecting the hydrocarbon intraperitoneally and isolated a second metabolite, which proved to be a quinone. Berenblum, Crowfoot, Holiday, and Schoental (3) have shown that BPX is identical with 8-hydroxy-3,4-benzpyrene, and that the quinonoid metabolite is 3,4-benzpyrene-5,8-quinone. From approximate analyses Berenblum and Schoental (2) consider that the greater part of the benzpyrene eliminated by the mouse appears in the excreta as one or the other of these two metabolites, accompanied by very small amounts of the unchanged hydrocarbon.

One of the present authors has described a method for the analysis of dibenzanthracene and its hydroxy-derivatives in tissues and excreta, based on the measurement of the ultraviolet absorption spectrum (18). The analysis, by this method, of the feces and urine of rats that had received large doses of dibenzanthracene, injected subcutaneously in olive oil, showed that over a period of several weeks only a small amount of the hydrocarbon could be accounted for in the excreta. Most of this eliminated material was present in the feces as the hydrocarbon, accompanied by traces only of 4′8′-dihydroxydibenzanthracene (19). Examination of the carcasses of rats that had been killed 8 weeks after they had received similar injections showed that large amounts of dibenzanthracene remained encapsulated in vesicles near the site of the injection (20). It was thus apparent that the subcutaneous route is unsuitable for quantitative metabolic studies. A method of injection is needed that will insure complete absorption from the point of injection and ready transport to the site of metabolic transformation. Attempts to inject dibenzanthracene intravenously as a colloidal solution in horse serum saturated with cholesterol (23) were not successful, as it was impossible to inject sufficient material in

* This investigation was aided by a grant from The International Cancer Research Foundation.

[1] These will be referred to as "dibenzanthracene" and "benzpyrene" respectively.

this manner. The intraperitoneal injection of a solution in tricaprylin proved very satisfactory, and a single dose of 10 mgm. in 2.5 ml. of the solvent could be introduced into the peritoneal cavity of a rat without any apparent harmful effects. Dibenzanthracene injected in this manner was completely absorbed after 12 days. Berenblum and Schoental (1) also have observed that benzpyrene can be injected by the same route and is rapidly absorbed.

TABLE I: THE ULTRAVIOLET ABSORPTION SPECTRUM OF 1,2,5,6-DIBENZANTHRACENE: WAVE LENGTHS AND INTENSITIES OF THE MAXIMA (SOLVENT ETHANOL)

| Determined Photographically | | | Determined Photoelectrically | | |
|---|---|---|---|---|---|
| | Log Em | $E^{1\%}_{1\ cm.}$ | | Log Em | $E^{1\%}_{1\ cm.}$ |
| 2740 | 4.68 | 1720 | 2735 | 4.70 | 1800 |
| 2780 | 4.68 | 1720 | 2770 | 4.73 | 1930 |
| 2875 | 4.98 | 3440 | 2880 | 5.00 | 3600 |
| 2970 | 5.24 | 6270 | 2970 | 5.25 | 6400 |
| 3200 | 4.29 | 704 | 3200 | 4.30 | 718 |
| 3330 | 4.23 | 612 | 3330 | 4.24 | 627 |
| 3485 | 4.13 | 486 | 3482 | 4.19 | 558 |
| 3650 | 2.70 | 18.1 | 3633 | 2.75 | 20.2 |
| 3740 | 2.98 | 34.3 | 3725 | 3.02 | 37.8 |
| 3835 | 2.68 | 17.3 | 3826 | 2.64 | 15.7 |
| 3930 | 3.06 | 41.5 | 3930 | 3.05 | 40.5 |
| 4′8′-DIHYDROXYDIBENZANTHRACENE (SOLVENT ETHANOL) | | | | | |
| 2375 | 4.80 | 1950 | 2375 | 4.74 | 1700 |
| 2780 | 4.62 | 1290 | 2780 | 4.60 | 1235 |
| 2980 | 4.88 | 2345 | 2980 | 4.84 | 2145 |
| 3090 | 4.82 | 2045 | 3080 | 4.81 | 2000 |
| 3240 | 4.36 | 710 | 3240 | 4.33 | 664 |
| 3380 | 4.28 | 582 | 3380 | 4.29 | 605 |
| 3540 | 4.35 | 695 | 3540 | 4.31 | 634 |
| 3645 | 3.69 | 158 | 3620 | 3.73 | 173 |
| 3840 | 3.99 | 315 | 3840 | 3.91 | 263 |
| 4030 | 4.15 | 437 | 4060 | 4.05 | 348 |

## METHODS, MATERIALS, AND RESULTS

### ANALYTICAL TECHNIC

The general principles of the analytical method have been outlined previously (18). The intensities of absorption of dibenzanthracene and 4′8′-dihydroxydibenzanthracene at the wave lengths of the absorption maxima have been redetermined, using a Beckman photoelectric spectrophotometer (9). The data obtained check fairly well with those recorded previously (17), when a photographic method of spectrophotometry was used (see Table I); in all calculations discussed in this and the following paper, the standard intensity data obtained with the photoelectric instrument have been employed, as this method is less liable to subjective errors than the photographic method, which involves the visual matching of the density of photographic plates.

Solutions of pure hydrocarbons can be analyzed quantitatively by the spectrographic method with an accuracy of about ± 5%. In extracts of tissues and excreta the accuracy is reduced because of uncertainties in the magnitude of the background absorption, which result from the presence of other absorbing components in the extracts. Although such extraneous absorption cannot be accurately evaluated, its significance can be estimated by comparison of the *shape* of the absorption curve of the extract with that of the pure hydrocarbon or metabolite; the region of the spectrum in which the extraneous absorption is at a minimum can be observed, and the hydrocarbon or metabolite content of the extract can then be calculated on the basis of the intensity of one of the maxima in this region of the spectrum (18). Data on the recovery of 1,2-benzanthracene added to rat liver and mouse liver tissue have been reported (18), and further data on the recovery of dibenzanthracene and 4′8′-dihydroxydibenzanthracene under various experimental conditions are included in Table II.

4′8′-Dihydroxydibenzanthracene is much less stable than dibenzanthracene, and some loss undoubtedly occurs during the preparation of the extracts of tissues and excreta. The results of some trial determinations are included in Table II. The recovery of the dihydric phenol, carried through the procedure for separation from the hydrocarbon, averaged 65 to 80 per cent. Recoveries of the same order were obtained when 4′8′-dihydroxydibenzanthracene was added to rat or rabbit feces and extracted with ether. The recovery is influenced considerably by the presence of peroxides in the ether used for extraction, and ether freshly distilled off solid sodium hydroxide was always employed. Such ether liberates no iodine on shaking with 5 per cent aqueous potassium iodide solution. The sodium salt of the phenol is less stable than the free hydroxy compound; as it is necessary to extract the phenolic material from the hydrocarbon with 0.1 *N* sodium hydroxide solution it is essential, for good recovery, that this stage of the separation be carried through as rapidly as possible.

## EXPERIMENTAL OBSERVATIONS

The preliminary experiments reported previously (19, 20) suggested that the free hydrocarbon and the dihydric phenol present in the excreta account for only a small part of the hydrocarbon lost from the body of the rat. The experiment that follows was designed to investigate this under more carefully controlled conditions, and to obtain some idea of the rate of elimination.

Nine rats (4 male and 5 female) were each injected intraperitoneally with 10 mgm. of dibenzanthracene dissolved in 2.5 ml. of synthetic tricaprylin.

TABLE II: SPECTROGRAPHIC ANALYSIS OF POLYNUCLEAR AROMATIC HYDROCARBONS, ETC.

(a) SOLUTIONS OF PURE HYDROCARBONS IN ETHANOL

| Hydrocarbon | Added | Found | Recovery |
|---|---|---|---|
| Anthracene | 8.6 mgm. | 7.8 mgm. | 92% |
| Pyrene | 18.2 " | 17.6 " | 97 |
| 1,2-Benzanthracene | 7.5 " | 7.9 " | 105 |
| Phenanthrene | 10.7 " | 10.9 " | 102 |

(b) DIBENZANTHRACENE CARRIED THROUGH THE PROCEDURE FOR SEPARATION FROM PHENOLIC SUBSTANCES

| Added | Found | Recovery |
|---|---|---|
| 1.3 mgm. | 1.2 mgm. | 93% |

(c) SEPARATION OF A MIXTURE OF DIBENZANTHRACENE AND 4′,8′-DIHYDROXYDIBENZANTHRACENE

Dibenzanthracene

| Added | Found | Recovery |
|---|---|---|
| 5.43 mgm. | 4.98 mgm. | 92% |
| 2.45 " | 2.48 " | 101 |
| 2.91 " | 3.08 " | 106 |
| 1.2 " | 1.0 " | 83 |

4′,8′-Dihydroxydibenzanthracene

| Added | Found | Recovery |
|---|---|---|
| 9.7 mgm. | 7.9 mgm. | 81% |
| 2.4 " | 1.7 " | 72 |
| 2.1 " | 1.3 " | 65 |
| 0.6 " | 0.45 " | 75 |

(d) 4′8′-DIHYDROXYDIBENZANTHRACENE MIXED WITH 40 GM. OF RAT FECES, EXTRACTED, AND PROCESSED

| Added | Found | Recovery |
|---|---|---|
| 6.8 mgm. | 4.1 mgm. | 60% |
| 1.99 " | 1.6 " | 81 |

(e) DIBENZANTHRACENE AND 4′8′-DIHYDROXYDIBENZANTHRACENE MIXED WITH 20 GM. OF RAT FECES, EXTRACTED, AND PROCESSED

Dibenzanthracene

| Added | Found | Recovery |
|---|---|---|
| 4.05 mgm. | 3.94 mgm. | 98% |

4′,8′-Dihydroxydibenzanthracene

| Added | Found | Recovery |
|---|---|---|
| 2.04 mgm. | 1.45 mgm. | 71% |

(f) DIBENZANTHRACENE AND 4′,8′-DIHYDROXYDIBENZANTHRACENE ADDED TO 40 GM. OF RABBIT FECES, EXTRACTED, AND PROCESSED

Dibenzanthracene

| Added | Found | Recovery |
|---|---|---|
| 6.1 mgm. | 6.1 mgm. | 100% |

4′,8′-Dihydroxydibenzanthracene

| Added | Found | Recovery |
|---|---|---|
| 9.7 mgm. | 7.6 mgm. | 78% |

The animals were kept in metabolism cages, and the urine and feces collected separately at 48-hour intervals for 14 days and stored at 5° C. The urine and feces were also collected from 2 control rats that received injections of tricaprylin only.

On the 14th day the rats were anesthetized, killed, and bled. The peritoneal cavities were opened and flushed out 3 times with ether; the ethereal washings were bulked and saved for spectrographic analysis. The bodies were autopsied and the following parts stored under ethanol: (a) blood, (b) livers, (c) gastrointestinal tracts with contents, (d) residual carcasses.

A gross examination of the organs showed that small white patches varying in size up to 4 × 12 mm. were present on the surfaces of the livers of all the animals. These patches fluoresced a brilliant blue in ultraviolet light. The pelvic and mesenteric fat showed a faint bluish-white fluorescence. The organs and viscera were otherwise grossly normal. No fluorescing patches were present on the livers of the control animals.

### PREPARATION OF ORGANS, ETC., FOR ANALYSIS

Each 48-hour batch of feces was ground with an equal weight of anhydrous sodium sulfate and extracted in a Soxhlet extractor with peroxide-free ether until fresh extracts were no longer fluorescent. The livers were treated in a similar manner. The urines were rendered faintly acid to Congo red paper with *N* hydrochloric acid and extracted with ether in a liquid/liquid extractor until all fluorescent material had been removed. During this extraction it was necessary to add more hydrochloric acid from time to time to maintain the urine sufficiently acid. The viscera and carcasses were minced finely and refluxed with ether to extract all fluorescing substances. No fluorescent material could be removed from the blood with ether.

The several ethereal extracts thus obtained above were evaporated to small bulk on a water bath at 50° C. in an atmosphere of nitrogen, and stored at 5° C. pending separation into neutral and phenolic fractions. The extracts were diluted to a convenient concentration with peroxide-free ether and the solutions shaken 3 times with normal sodium carbonate; the aqueous alkaline extracts were rejected. The ethereal phase was next extracted repeatedly with 0.1 *N* sodium hydroxide solution until fresh extracts no longer exhibited a yellow fluorescence. If necessary the solutions were centrifuged to break emulsions. After the complete extraction of the dihydric phenol the ethereal phase was set aside, and the alkaline solution containing the sodium salt of the dihydric

phenol washed several times with ether, covered immediately with a layer of fresh ether, and saturated with carbon dioxide for one hour with frequent shaking. The phenolic material passed into the ether; the yellow fluorescence of the aqueous phase disappeared, and blue fluorescence developed in the organic layer. The ethereal phase was washed several times with water, dried with anhydrous sodium sulfate, and the solvent removed in a stream of nitrogen on a water bath at 50° C. The residue was dried to constant weight in an evacuated desiccator at room temperature and dissolved in a known volume of ethanol (usually 10 ml.) for spectrographic analysis.

The neutral fractions of the feces and urines were washed with water, dried with anhydrous sodium sulfate, and prepared for spectrographic analysis in the same manner as the phenolic fractions described above. The neutral fractions of the feces contained carotenoid pigments, but were free from chlorophyll.

The neutral fractions from the livers, gastrointes-

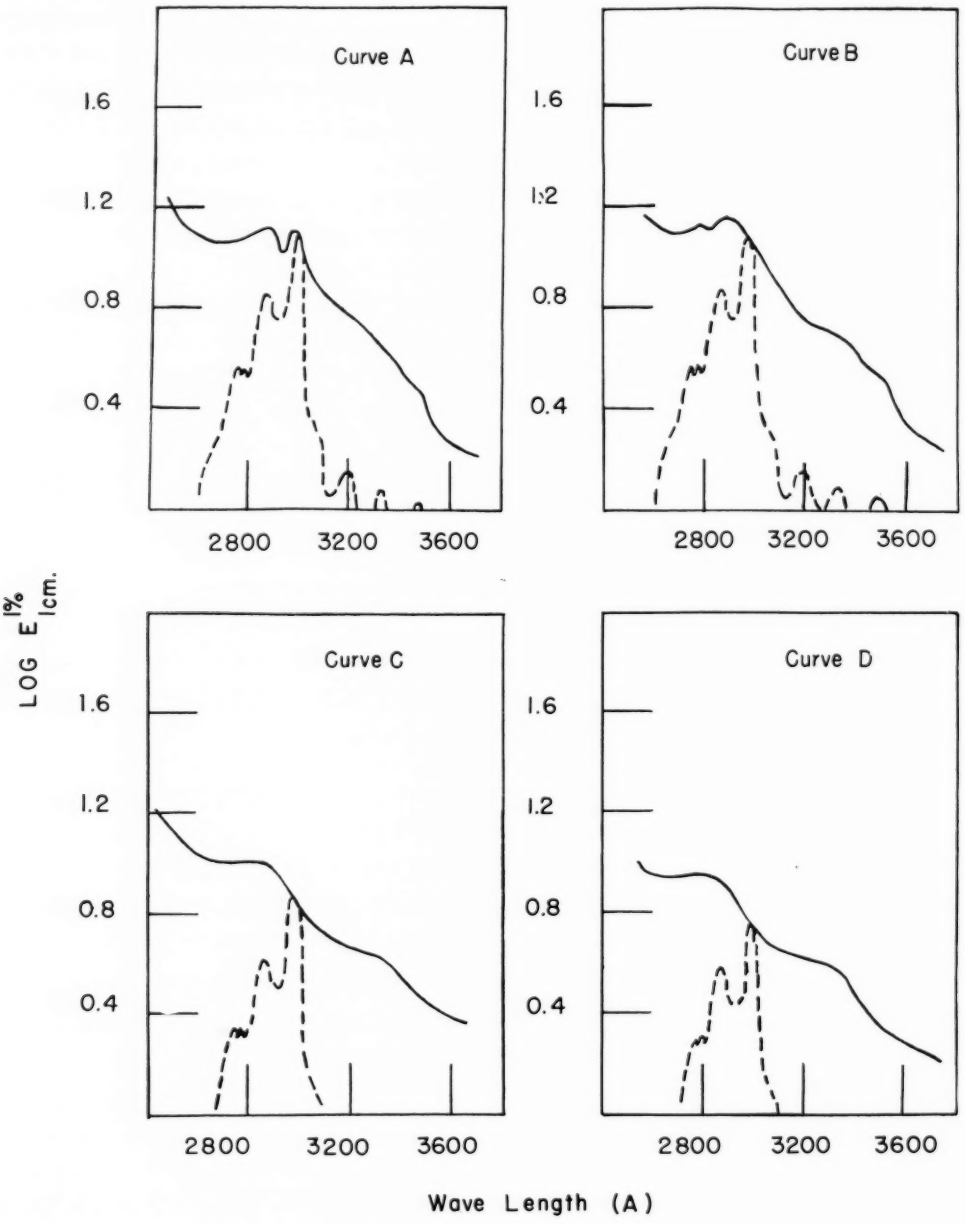


FIG. 1.—Continuous line ———— ultraviolet absorption spectra of neutral fractions of feces. Dash line – – – – – – theoretical curve of dibenzanthracene. (Solvent ethanol.)

| | | | | |
|---|---|---|---|---|
| Curve | A | Fraction | I | 0-2 days |
| " | B | " | III | 4-6 " |
| " | C | " | V | 8-10 " |
| " | D | " | VII | 12-14 " |

tinal tracts, and residual carcasses contained much fatty material. They were dissolved in ethanol and refluxed with alcoholic potassium hydroxide solution for 6 to 18 hours, diluted with water, and the nonsaponifiable material was extracted with ether. The ethereal phases were washed with water, dried with anhydrous sodium sulfate, and prepared for spectrographic analysis as described above.

characterized unequivocally as the spectrum of dibenzanthracene, although the strong blue fluorescence of all the extracts suggested that they contained some of the unchanged hydrocarbon. The daily excretions, based on the intensity at 2,970 A., are given in Table III, from which it may be calculated that 5.4 mgm. were excreted over the 14-day period. This figure is undoubtedly much too high. There was no evidence of

TABLE III: NEUTRAL EXTRACTS OF FECES

| Fraction | Time | Wt. of feces | Wt. of extract | $E^{1\%}_{1\ cm.\ 2970\ A.}$ | Wt. of dibenzanthracene | Agreement * with shape of theoretical curve |
|---|---|---|---|---|---|---|
| I | 0-2 days | 74 gm. | 602 mgm. | 12.9 | 1.2 mgm. | ++ |
| II | 2-4 " | 299 " | 451 " | 7.1 | 0.5 " | + |
| III | 4-6 " | 369 " | 497 " | 12.0 | 0.9 " | + |
| IV | 6-8 " | 83 " | 649 " | 8.3 | 0.8 " | + |
| V | 8-10 " | 78 " | 570 " | 7.9 | 0.7 " | + |
| VI | 10-12 " | 77 " | 657 " | 6.6 | 0.7 " | + |
| VII | 12-14 " | 81 " | 672 " | 6.3 | 0.6 " | + |
| | | | | | Total 5.4 mgm. | |

* See footnote to Table V.

TABLE IV: PHENOLIC EXTRACTS OF FECES

| Fraction | Time | Wt. of fraction | $E^{1\%}_{1\ cm.\ 4060\ A.}$ | Wt. of dihydric phenol | Agreement * with shape of theoretical curve |
|---|---|---|---|---|---|
| I | 0-2 days | 43 mgm. | 1.8 | 0.22 mgm. | +++ |
| II | 2-4 " | 64 " | 0.85 | 0.15 " | +++ |
| III | 4-6 " | 51 " | 2.5 | 0.36 " | +++ |
| IV | 6-8 " | 38 " | 2.2 | 0.24 " | +++ |
| V | 8-10 " | 81 " | 0.7 | 0.16 " | ++ |
| VI | 10-12 " | 50 " | 1.2 | 0.17 " | ++ |
| VII | 12-14 " | 48 " | 1.1 | 0.15 " | ++ |
| | | | | Total 1.45 mgm. | |

* See footnote to Table V.

TABLE V: NEUTRAL EXTRACTS OF CARCASSES, ETC.

| Fraction | Wt. of extract | $E^{1\%}_{1\ cm.\ 2970\ A.}$ | Wt. of dibenzanthracene | Agreement * with shape of theoretical curve |
|---|---|---|---|---|
| Livers | 1.120 gm. | 47 | 8.3 mgm. | ++++ |
| Gastrointestinal tracts with contents | 0.508 " | 63 | 5.0 " | ++++ |
| Residual carcasses | 43.3 " | 1.5 | 10.5 " | ++++ |
| Ethereal washings from peritoneal cavity | 0.012 " | 21 | 0.04 " | ++++ |
| Blood | (No fluorescent extracts—not analyzed) | | | |
| | | | Total 23.8 mgm. | |

* The deviation from the shape of the theoretical curve is caused by general absorption of nonspecific components in the extracts (18). In the cases where the agreement is represented by ++++ the analytical data can be accepted as quantitative within the tolerances of Table II. Where the agreement is represented by +++, ++, or + the analytical figures are progressively less accurate. In the case of dibenzanthracene analyses these data set an upper limit to the hydrocarbon content.

## RESULTS

### FECES

The neutral fractions of the fecal extracts all gave spectra with a maximum between 2,800-3,000 A., not present in the spectra of similar extracts from the feces of control rats (Curves 1A-1D). Only in the case of the first 48-hour fecal batches could this be any pronounced peak in the excretion rate over the time covered by these measurements.

The phenolic fractions of the fecal extracts all exhibited a well-characterized spectrum of 4′8′-dihydroxydibenzanthracene (Curves 2A-2D and Table IV); but the actual amounts of 4′8′-dihydroxydibenzanthracene detected in the feces were small. The rate of excretion was fairly uniform over the period

under study, the total amount determined being 1.45 mgm.

### Urine

The neutral fractions of the 48-hour urine extracts did not show any spectrographic evidence of the presence of dibenzanthracene. The extracts were bulked and chromatographed on alumina from hexane solution. A blue fluorescent zone formed on the column. The zone was eluted with hexane containing 25 per cent of ether and yielded 4 mgm. of a fluorescent gum that gave a well-characterized dibenzanthracene spectrum from which the content of hydrocarbon was calculated to be 0.9 mgm.

None of the phenolic extracts of the 48-hour urine samples showed spectrographic evidence of 4′8′-dihydroxydibenzanthracene. All possessed a well-developed maximum near 2,800 A., but this was present also in similar extracts from the urines of the control animals. The phenolic extracts were bulked and chromatographed from benzene solution on alumina to yield two faint blue fluorescent zones, one of which was eluted with hexane containing 25 per cent of ether and the other with ethanol. The material from neither zone gave a spectrum with characteristic structure. It is surmised that the more easily eluted zone was due to traces of dibenzanthracene not removed by separation with alkali, and the more strongly adsorbed zone to traces of 4′8′-dihydroxydibenzanthracene. The amounts of fluorescing materials present must have been exceedingly small.

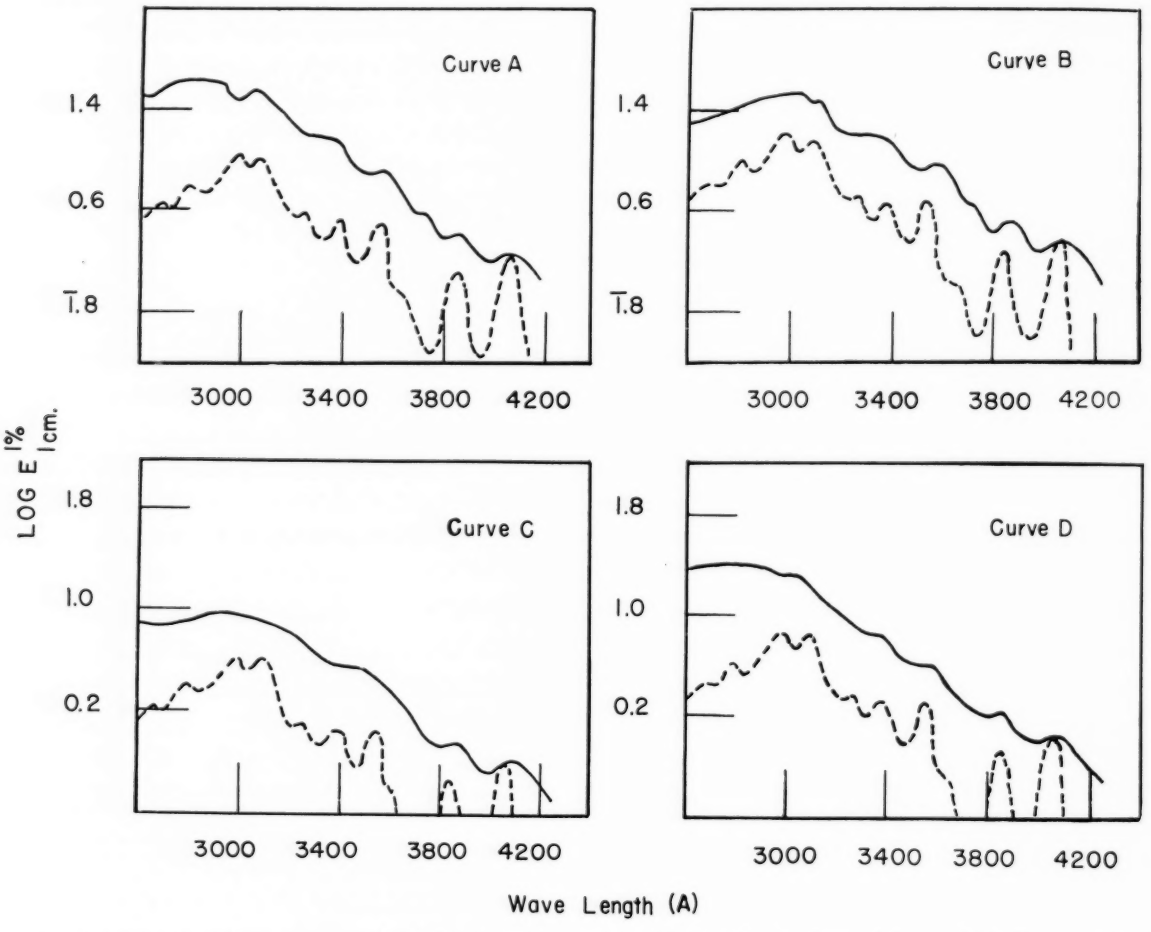


Fig. 2.—Continuous line ———— ultraviolet absorption spectra of phenolic fractions of feces. Dash line – – – – – – theoretical curve of 4′,8′-dihydroxydibenzanthracene. (Solvent ethanol.)

| | | | | |
|---|---|---|---|---|
| Curve | A | Fraction | I | 0-2 days |
| " | B | " | III | 4-6 " |
| " | C | " | V | 8-10 " |
| " | D | " | VII | 12-14 " |

### Organs

The neutral fractions of the several parts of the carcasses gave spectra with excellently defined dibenzanthracene structure (Curves 3A-3D). The dibenzanthracene contents of the extracts calculated from

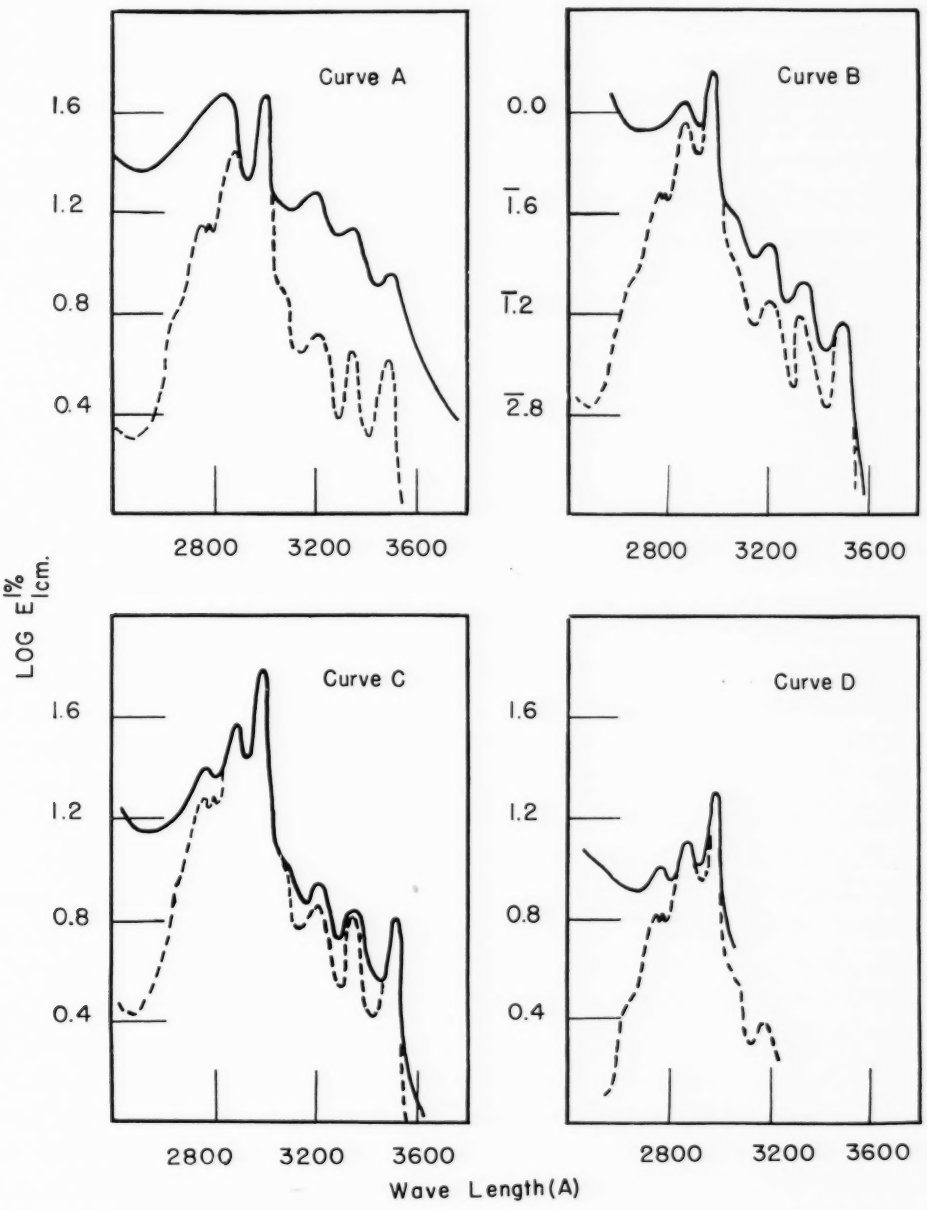


Fig. 3.—Continuous line ———— ultraviolet absorption spectra of neutral fractions of parts of carcasses, etc. Dash line - - - - - - theoretical curve of dibenzanthracene. (Solvent ethanol.)

Curve A Liver.
" B Residual carcasses.
" C Gastrointestinal tract with contents.
" D Ether washings from the peritoneal cavity.

these curves should be fairly accurate (Table V). The ether washings from the peritoneal cavities also gave a good dibenzanthracene spectrum, although the amount of hydrocarbon present was only 0.04 mgm.

No 4′8′-dihydroxydibenzanthracene was detected in any of the phenolic extracts from the parts of the carcasses.

TABLE VI: DISTRIBUTION OF DIBENZANTHRACENE AND 4′8′-DIHYDROXYDIBENZANTHRACENE

| | Dibenzanthracene | 4′8′-Dihydroxy-dibenzanthracene |
|---|---|---|
| Feces | Less than 5.4 mgm. | 1.45 mgm. |
| Urines | 0.9 " | trace |
| Livers | 8.3 " | nil |
| Gastrointestinal tracts with contents | 5.0 " | nil |
| Residual carcasses | 10.5 " | nil |
| Blood | nil | nil |
| Washings from peritoneal cavity | 0.04 " | nil |
| Total recovered | 30.1 mgm. | 1.45 mgm. |
| Total injected | 90 " | |
| Percentage excreted | Less than 6% | 1.6% |
| Percentage recovered | Less than 33% | 1.6% |

## DISCUSSION

Consideration of Tables III, IV, V, and VI shows that 14 days after injection a relatively large amount of dibenzanthracene remains in the carcasses in an unchanged condition. Curves 3A, 3B, and 3C show that the neutral fractions of the extracts from the livers, gastrointestinal tracts, and residual carcasses gave well-characterized spectra; the total amount of hydrocarbon present in these organs was determined as 23.8 mgm., or 26 per cent of the amount injected. No phenolic metabolite was detected in any of these tissue fractions. The tissues of the gastrointestinal tracts were analyzed together with the contents of the gut, and the 5 mgm. of dibenzanthracene recovered from these organs were probably associated with the intestinal tissue rather than the contents, in view of the very small amounts recovered from the feces during the entire 14 days of the experiment.

The amounts of hydrocarbon and phenolic metabolite excreted in the feces were small, and there is no indication of any pronounced peak in the excretion rate from day to day. In this the results are in strong contrast with those obtained with the rabbit, discussed in the succeeding paper (21). The urine contained traces only of hydrocarbon and phenolic metabolite, and the possibility cannot be excluded that much of this may be attributed to incomplete separation from feces in the metabolism cages.

Naphthalene (4) is mainly excreted as the mercapturic acid derivative in the urine, and Boyland and Levi (5—7) have shown that 1,2-dihydroxydihydroanthracene can be isolated in yields up to 100 mgm. per liter from the urine of rats that have been fed a diet containing anthracene. Dobriner, Rhoads, and Lavin (16) detected 4′8′-dihydroxydibenzanthracene spectrographically in the *urine* of rats injected subcutaneously with dibenzanthracene, but these workers isolated the crystalline phenolic metabolite only from *combined excreta*. Our observations substantiate the earlier suggestions that only minute amounts of dibenzanthracene are excreted in the urine. With increasing molecular weight the elimination of aromatic hydrocarbons in the urine appears to become less effective; this may be due to the difficulty of solubilizing them sufficiently. While it is possible that part of the hydrocarbon may be degraded to smaller fragments by ring rupture, and such fragments may be present in the urine in quantity, this has not yet been demonstrated in the case of any polynuclear aromatic hydrocarbon.

During the last few years intensive investigations have been carried out in several laboratories on the detection and characterization of traces of steroid metabolites in urine, and search is being made for endogenous carcinogenic agents in human urine. Perhaps more consideration should be given to the possibility that the main path for the elimination of some steroids might be fecal rather than urinary; particularly might this be true of steroids containing few water-solubilizing groups and partly aromatized ring structures. It is among steroids of this type that one might expect to find carcinogenic agents.

Of the 90 mgm. of dibenzanthracene injected, 23.8 mgm. were retained unchanged in the body, less than 5.5 mgm. were excreted unchanged, and only 1.45 mgm. of 4′8′-dihydroxydibenzanthracene was detected. This latter figure might be low because of decomposition of the unstable metabolite during processing and analysis. Even so, almost two-thirds of the hydrocarbon are not accounted for, and decomposition of 4′8′-dihydroxydibenzanthracene can hardly make up more than a small part of the discrepancy. It is thus reasonable to assume that most of the injected dibenzanthracene is converted to other derivatives. Berenblum and Schoental (2) isolated 3,4-benzpyrene-5,8-quinone from the feces of rats injected intraperitoneally with benzpyrene, and it is possible that parts of the dibenzanthracene may also be excreted as a quinone or oxidized to a quinone after excretion. This may not be detected spectrographically, since the spectra of quinones do not normally exhibit the well-developed fine structure maxima characteristic of aromatic hydrocarbons and phenols; neither do they fluoresce.

A similar series of experiments has been carried out on the rabbit (21), and the results of the ex-

periments described here on metabolism by the rat will be discussed further in relation to the rabbit experiments in the following paper.


## SUMMARY

Spectrographic analyses have been made of the organs and excreta of 9 rats, each of which received an intraperitoneal injection of 10 mgm. of dibenzanthracene dissolved in tricaprylin. After 14 days the absorption of the hydrocarbon from the peritoneal cavity was almost complete. The feces, collected at 48-hour intervals, contained less than 5.4 mgm. of dibenzanthracene and about 1.5 mgm. of 4′8′-dihydroxydibenzanthracene. Only traces of hydrocarbon or phenolic metabolite were excreted in the urine, and 25 per cent of the hydrocarbon remained in the carcasses in an unchanged condition after 14 days. The phenolic metabolite was detected only in the excreta. The accuracy of the analytical data varied from one extract to another, because of variations in the intensity of the background absorption. It is considered that whereas the data for the hydrocarbon remaining in the organs and carcasses are fairly accurate, the figures given for the hydrocarbon and phenolic material in the fecal extracts should be regarded as estimates only.

The greater part of the hydrocarbon was not accounted for and was presumably metabolized to some other product, which was not detected by the analytical methods employed.



## ACKNOWLEDGMENT

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## REFERENCES


1. Berenblum, I., and Schoental, R. The Rate of Disappearance of 3:4-Benzpyrene from the Mouse after Subcutaneous and Intraperitoneal Injection. Biochem. J., **36**: 92-97. 1942.
2. Berenblum, I., and Schoental, R. The Metabolism of 3,4-Benzpyrene in Mice and Rats. I. The Isolation of a Hydroxy and a Quinone Derivative, and a Consideration of Their Biological Significance. Cancer Research, **3**:145-150. 1943.
3. Berenblum, I., Crowfoot, D., Holiday, E. R., and Schoental, R. The Metabolism of 3,4-Benzpyrene in Mice and Rats. II. The Identification of the Isolated Products as 8-Hydroxy-3,4-Benzpyrene and 3,4-Benzpyrene-5,8-Quinone. Cancer Research **3**:151-158. 1943.
4. Bourne, M. C., and Young, L. The Metabolism of Naphthalene in Rabbits. Biochem. J., **28**:803-808. 1934.
5. Boyland, E., and Levi, A. A. Metabolism of Polycyclic Compounds. I. Production of Dihydroxydihydroanthracene from Anthracene. Biochem. J., **29**:2679-2683. 1935.
6. Boyland, E., and Levi, A. A. Metabolism of Polycyclic Compounds. II. Production of Dihydroxydihydroanthraceneglycuronic Acid from Anthracene. Biochem. J., **30**: 728-731. 1936.
7. Boyland, E., and Levi, A. A. Metabolism of Polycyclic Compounds. III. Anthrylmercapturic Acid. Biochem. J., **30**:1225-1227. 1936.
8. Boyland, E., Levi, A. A., Mawson, E. H., and Roe, E. Metabolism of Polycyclic Compounds. IV. Production of a Dihydroxy-1:2:5:6-Dibenzanthracene from 1:2:5:6-Dibenzanthracene. Biochem. J., **35**:184-191. 1941.
9. Cary, H. H., and Beckman, A. O. A Quartz Photoelectric Spectrophotometer. J. Optic. Soc. America, **31**:682-689. 1941.
10. Cason, J., and Fieser, L. F. Synthesis of 4′,8′-Dihydroxy-1,2,5,6-Dibenzanthracene and Its Relation to Products of Metabolism of the Hydrocarbon. J. Am. Chem. Soc., **62**:2681-2687. 1940.
11. Chalmers, J. G. The Elimination of 3:4-Benzpyrene and Other Polycyclic Hydrocarbons from the Mouse. Biochem. J., **32**:271-278. 1938.
12. Chalmers, J. G. Elimination of 3:4-Benzpyrene from the Rat. Biochem. J., **34**:678-684. 1940.
13. Chalmers, J. G., and Crowfoot, D. The Elimination of 3:4-Benzpyrene from the Animal Body after Subcutaneous Injection. II. Changed Benzpyrene. Biochem. J., **35**: 1270-1275. 1941.
14. Chalmers, J. G., and Kirby, A. H. M. The Elimination of 3:4-Benzpyrene from the Animal Body after Subcutaneous Injection. I. Unchanged Benzpyrene. Biochem. J., **34**:1191-1195. 1940.
15. Dobriner, K., Rhoads, C. P., and Lavin, G. I. Conversion of 1,2,5,6-Dibenzanthracene by Rabbits, Rats, and Mice. Significance in Carcinogenesis of this Conversion. Proc. Soc. Exper. Biol. & Med., **41**:67-69. 1939.
16. Dobriner, K., Rhoads, C. P., and Lavin, G. I. The Spectroscopic Study of Biological Extracts. II. The Detection, Isolation, and Biological Effects of the Metabolites of 1,2,5,6-Dibenzanthracene. Cancer Research, **2**:95-107. 1942.
17. Jones, R. N. The Ultraviolet Absorption Spectra of Some Derivatives of 1,2-Benzanthracene. J. Am. Chem. Soc., **62**:148-152. 1940.
18. Jones, R. N. The Spectrographic Analysis of Carcinogenic Hydrocarbons and Metabolites. I. Introduction. Cancer Research, **2**:237-244. 1942.
19. Jones, R. N. The Spectrographic Analysis of Carcinogenic Hydrocarbons and Metabolites. II. Determination of 1,2,5,6-Dibenzanthracene and 4′,8′-Dihydroxy-1,2,5,6-Dibenzanthracene in Rat Extreta. Cancer Research, **2**: 245-251. 1942.
20. Jones, R. N. The Spectrographic Analysis of Carcinogenic Hydrocarbons and Metabolites. III. Distribution of 1,2,5,6-Dibenzanthracene in Rats Following Subcutaneous Injection in Olive Oil. Cancer Research, **2**:252-255. 1942.
21. Jones, R. N., Dunlap, C. E., and Gogek, C. J. The Spectrographic Analysis of Carcinogenic Hydrocarbons and Metabolites. V. The Elimination of 1,2,5,6-Dibenzanthracene from the Rabbit. Cancer Research, **4**:218-226. 1944.
22. Levi, A. A., and Boyland, E. The Production of Dihydroxy-1:2:5:6-Dibenzanthracene from 1:2:5:6-Dibenzanthracene by Rabbits. Chem. and Indust., **15**:446. 1937.
23. Lorenz, E., and Andervont, H. B. Note on the Preparation of Dispersions of Carcinogenic Hydrocarbons in Serum. Am. J. Cancer, **26**:783-786. 1936.
24. Peacock, P. R. Evidence Regarding the Mechanism of Elimination of 1:2-Benzpyrene, 1:2:5:6-Dibenzanthracene, and Anthracene from the Bloom-Stream of Injected Animals. Brit. J. Exper. Path., **17**:164-172. 1936.

# The Spectrographic Analysis of Carcinogenic Hydrocarbons and Metabolites

## V. The Elimination of 1,2,5,6-Dibenzanthracene from the Rabbit*

R. Norman Jones, Ph.D., C. E. Dunlap, M.D., and C. J. Gogek, B.Sc.

*(From The Chemistry Department, Queen's University, Kingston, Canada; The Converse Memorial Laboratory, Harvard University, Cambridge, Massachusetts; and The Department of Pathology, Harvard Medical School, Boston, Massachusetts)*

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The rabbit has generally been regarded as resistant to the carcinogenic action of those polynuclear aromatic hydrocarbons that are potent carcinogenic agents in rats and mice. The literature of hydrocarbon carcinogenesis in rabbits has been reviewed in a recent article by Syverton, Berry, and Dascomb (11), who have observed that although the rabbit is relatively immune to the carcinogenic activity of injected 1,2,5,6-dibenzanthracene [1] and 3,4-benzpyrene,[1] this is not true for 20-methylcholanthrene, which is active in both western cotton-tail and common domestic rabbits.

Comparative studies of the metabolism of aromatic hydrocarbons by rats and rabbits show that the elimination products may differ significantly in the two species. Boyland and Levi (2) found that whereas anthracene, administered orally to rats, is excreted in the urine as *levo*-1,2-dihydroxy-1,2-dihydroanthracene, the rabbit excretes an optical isomer of the same compound, *dextro*-1,2-dihydroxy-1,2-dihydroanthracene. Dibenzanthracene is excreted by the rat and mouse as 4′8′-dihydroxydibenzanthracene (4, 7, 8, 9), but the rabbit excretes a structural isomer (3, 7, 8) in which the positions of attachment of the hydroxyl groups have not yet been established.

A comparison of the elimination process in the two animals has been made by Dobriner, Rhoads, and Lavin (8), who have reported that rats and mice excrete relatively large amounts of unchanged dibenzanthracene as compared with rabbits, but convert only a small amount of subcutaneously injected dibenzanthracene to the phenolic derivative. In this article we report the results of some quantitative studies of the elimination of dibenzanthracene and its phenolic metabolite from rabbits that received intraperitoneal injections of the hydrocarbon in solution in tricaprylin. The conditions parallel closely those employed in the investigation of the elimination of dibenzanthracene from the rat, discussed in a previous paper (9).

* This investigation was aided by a grant from The International Cancer Research Foundation.

[1] These compounds will subsequently be referred to as "dibenzanthracene" and "benzpyrene" respectively.

### METHODS, MATERIALS, AND RESULTS

#### Analytical Technic

The analytical procedures employed were described in a previous paper (9). The absorption spectra of concentrates of the phenolic metabolite from the rabbit (Figs. 2 and 3) possess maxima at the same wave lengths as those reported by Boyland, Levi, Mawson, and Roe (3), and the spectrum differs from that of 4′8′-dihydroxydibenzanthracene excreted by the rat. The concentrations of the dihydric phenol in the phenolic fractions from the rabbit extracts were calculated on the basis of intensity data interpolated from the absorption curve of Boyland, Levi, Mawson, and Roe (3) for the purified metabolite (Table I).

Table I: The Ultraviolet Absorption Spectrum of the Phenolic Metabolite of 1,2,5,6-Dibenzanthracene Excreted by the Rabbit. (Solvent Ethanol)

| Wave length of maximum | Log $E_m$ | $E^{1\%}_{1\ cm.}$ |
|---|---|---|
| 2300 A. | 4.65 | 1445 |
| 2820 | 4.50 | 1020 |
| 2930 | 4.82 | 2130 |
| 3050 | 4.95 | 2880 |
| 3300 | 4.05 | 365 |
| 3430 | 4.05 | 365 |
| 3600 | 4.10 | 410 |
| 3850 | 3.35 | 73 |
| 4070 | 3.35 | 73 |

#### EXPERIMENTAL OBSERVATIONS

##### A. Elimination of Dibenzanthracene and Dihydroxydibenzanthracene over a Twenty-Eight Day Period

One female rabbit was injected intraperitoneally with 150 mgm. of dibenzanthracene in 35 ml. of

synthetic tricaprylin. The animal was placed in a metabolism cage and the urine and feces were collected separately for two 2 day periods and at subsequent 4 day intervals. Urine and feces were collected and bled. The peritoneal cavity was opened and washed three times with ether, and the ether washings were collected and stored. The following parts of the carcass were dissected out and stored under

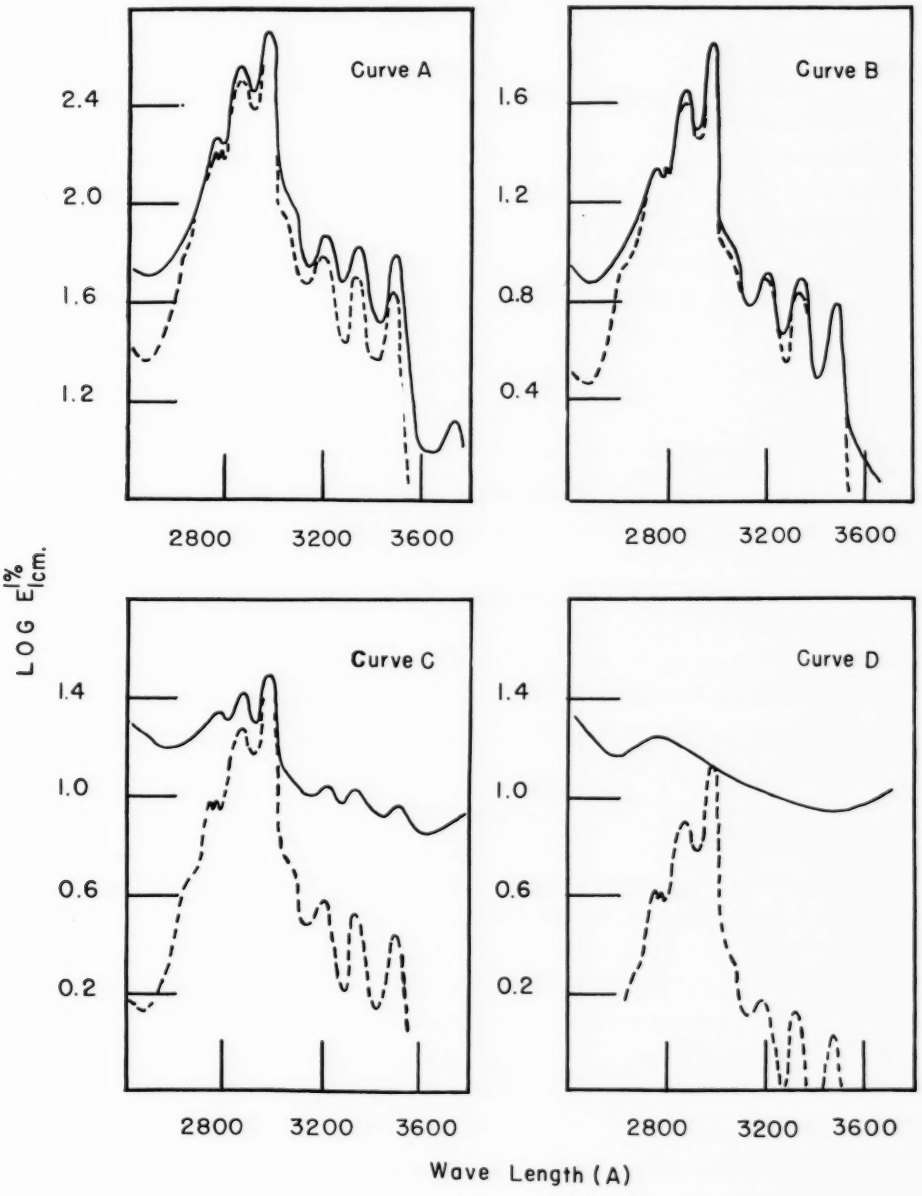


FIG. 1.—28 day rabbit. Continuous line ———— ultraviolet absorption spectra of neutral fractions of feces. Dash line - - - - - - theoretical curve of dibenzanthracene. (Solvent ethanol.)

| | | | | |
|---|---|---|---|---|
| Curve | A | Fraction III | 4-8 | days |
| " | B | " IV | 8-12 | " |
| " | C | " V | 12-16 | " |
| " | D | " VI | 16-20 | " |

also from a control rabbit that had received an injection of tricaprylin only. No harmful effects followed the injection except for temporary constipation in both the experimental and control animal. This condition corrected itself after 4 days.

On the 28th day the experimental animal was killed and bled. The peritoneal cavity was opened and washed three times with ether, and the ether washings were collected and stored. The following parts of the carcass were dissected out and stored under ethanol until prepared for spectrographic analysis: (a) liver, (b) gastrointestinal tract with contents, (c) gall bladder with contents, (d) residual carcass, (e) blood.

*Preparation of extracts for analysis.*—The several parts of the carcass, as well as the 2 day and 4 day

urines and feces were extracted with ether and separated into neutral and phenolic fractions as described in a previous publication (9). The neutral fractions of the feces contained much green pigment, which was destroyed before analysis by saponification with alcoholic potassium hydroxide solution. The neutral fractions of the liver, gastrointestinal tract, and carcass were also saponified to remove fatty material.

RESULTS

Analytical data for the 28 day rabbit are given in Tables II to VII, and for the 4 day rabbit in Table VIII.

From the data presented in Table VII, which summarizes the findings for the 28 day rabbit, it will be seen that the animal eliminated 42 per cent of the injected hydrocarbon, either in the unchanged

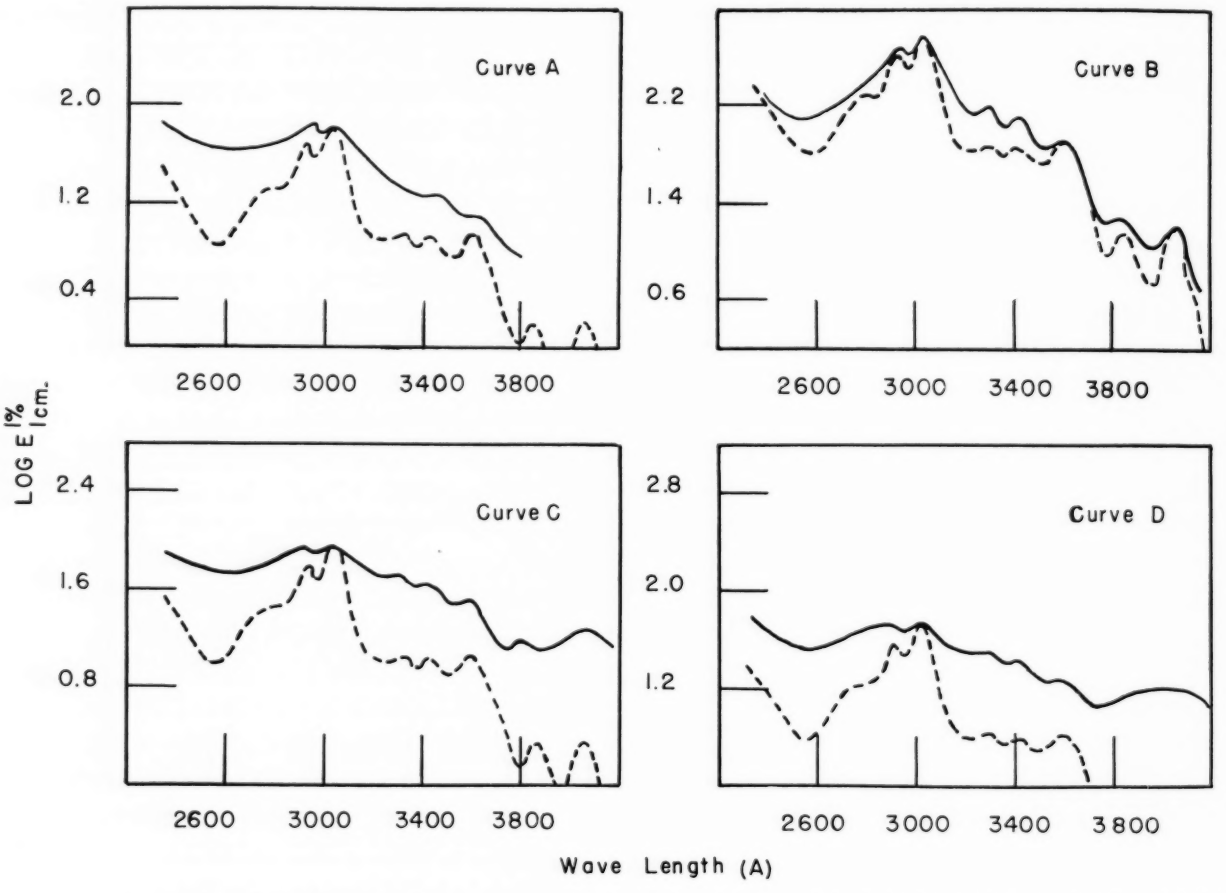


FIG. 2.—28 day rabbit. Continuous line ———— ultraviolet absorption spectra of phenolic fractions of feces. Dash line - - - - - - theoretical curve of pure phenolic metabolite. (Solvent ethanol.)

| | | | | |
|---|---|---|---|---|
| Curve | A | Fraction | III | 4-8 days |
| " | B | " | IV | 8-12 " |
| " | C | " | V | 12-16 " |
| " | D | " | VI | 16-20 " |

### B. Elimination of Dibenzanthracene and Dihydroxydibenzanthracene over a 4 Day Period

In a second experiment a rabbit was injected with 150 mgm. of dibenzanthracene in the manner described above; feces and urine were collected separately for 4 days after which the animal was killed, bled, and the following carcass fractions dissected out and processed in the usual manner: (a) liver, (b) contents of cecum and large intestine, (c) contents of stomach and small intestine, (d) tissue of gastrointestinal tract, (e) blood, (f) gall bladder with contents, (g) residual carcass.

form or as a phenolic derivative, and that only traces remained in the carcass. This picture differed considerably from that shown by the rat, where, after 14 days, only small amounts of the hydrocarbon had been eliminated in a form detectable spectroscopically, and much remained in the carcasses.

The neutral fractions of the fecal extracts contained large quantities of unchanged dibenzanthracene. The rate of excretion of the hydrocarbon in the feces reached a very pronounced maximum between the eighth and 12th day after injection, but in connection with this it must be observed that during the first

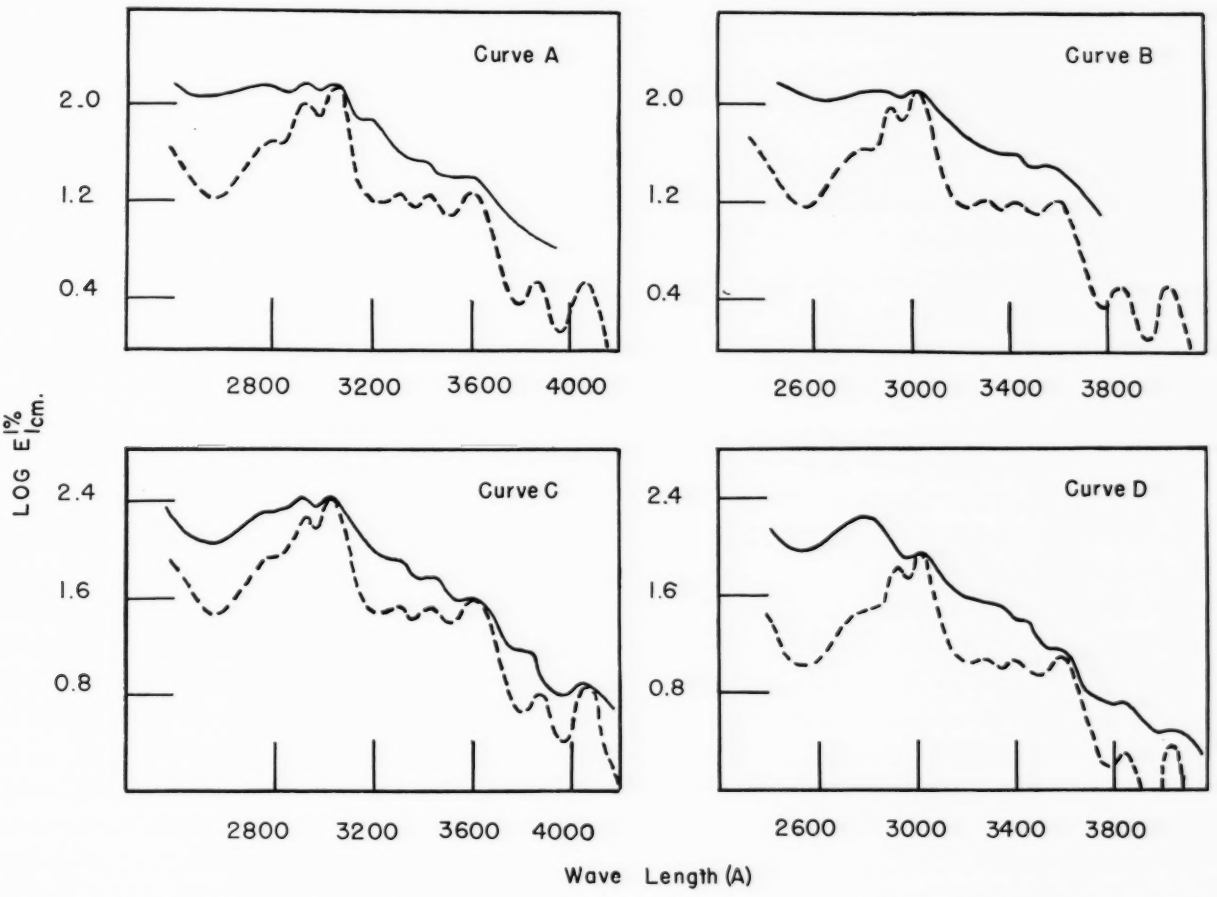


FIG. 3.—28 day rabbit. Continuous line ———— ultraviolet absorption spectra of phenolic fractions of urine. Dash line - - - - - - theoretical curve of pure phenolic metabolite. (Solvent ethanol.)

| | | | | |
|---|---|---|---|---|
| Curve | A | Fraction | I | 0-2 days |
| " | B | " | III | 4-8 " |
| " | C | " | IV | 8-12 " |
| " | D | " | V | 12-16 " |

TABLE II: 28 DAY RABBIT. NEUTRAL EXTRACTS OF FECES

| Fraction | Time | Wt. of feces | Wt. of extract | $E^{1\%}_{1\ cm.\ 2970\ A.}$ | Wt. of dibenzanthracene | Agreement * with shape of theoretical curve |
|---|---|---|---|---|---|---|
| I | 0-2 days | 0 gm. | — | — | — | — |
| II | 2-4 " | 15 " | (extract not fluorescent) | | — | — |
| III | 4-8 " | 15 " | 0.024 gm. | 575 | 1.7 mgm. | ++++ |
| IV | 8-12 " | 70 " | 3.490 " | 73 | 40.0 " | ++++ |
| V | 12-16 " | 110 " | 1.060 " | 31.7 | 5.3 " | +++ |
| VI | 16-20 " | 110 " | 1.220 " | 13.6 | (2.7) " | + |
| VII | 20-24 " | 89 " | 1.380 " | 7.3 | (1.6) " | + |
| VIII | 24-28 " | 216 " | (not analyzed) | | — | |
| | | | | Total | 51.3 mgm. | |
| | | | | | (34% of wt. injected). | |

* See footnote to Table VIII.

2 days the animal put out no feces, and the amounts put out between the second and fourth, and the fourth and eighth day were also small. The 4 day rabbit had already eliminated 5 mgm. of dibenzanthrecene in the feces, and a further 35.5 mgm. were present in the contents of the intestinal tract. These results suggest that excretion into the intestinal tract occurred very rapidly after injection.

In Table III the concentrations of the phenolic metabolite in successive 48 hour and 96 hour fecal fractions are evaluated. The rate of excretion parallels that of the hydrocarbon, and an intense peak was observed between the eighth and 12th day, after which the rate of excretion fell off very rapidly. After the 16th day there was practically no phenolic metabolite to be detected spectrographically in the feces (see Curves IIa-IId). The amounts of phenolic metabolite excreted were very much greater than those found in

TABLE III: 28 DAY RABBIT. PHENOLIC EXTRACT OF FECES

| Fraction | Time | Wt. of extract | $E^{1\%}_{1\ cm.,\ 3050\ A.}$ | Wt. of phenolic metabolite | Agreement * with shape of theoretical curve |
|---|---|---|---|---|---|
| I | 0-2 days | — | (no feces) | — | -- |
| II | 2-4 " | (Extract not fluorescent) | | — | — |
| III | 4-8 " | 2.5 mgm. | 71.0 | 0.06 mgm. | +++ |
| IV | 8-12 " | 39 " | 602 | 8.2 " | ++++ |
| V | 12-16 " | 88 " | 93 | 2.8 " | ++++ |
| VI | 16-20 " | 33 " | 60 | 0.7 " | ++ |
| VII | 20-24 " | 50 " | 26 | 0.5 " | + |
| VIII | 24-28 " | (Extract not analyzed) | | — | — |
| | | | | Total 11.3 mgm. | |

* See footnote to Table VIII.

TABLE IV: 28 DAY RABBIT. PHENOLIC EXTRACT FROM URINES

| Fraction | Time | Wt. of extract | $E^{1\%}_{1\ cm.,\ 3050\ A.}$ | Wt. of phenolic metabolite | Agreement * with shape of theoretical curve |
|---|---|---|---|---|---|
| I | 0-2 days | 3 mgm. | 166 | 0.16 mgm. | +++ |
| II | 2-4 " | 1 " | 100 | 0.04 " | ++ |
| III | 4-8 " | 1 " | 131 | 0.04 " | ++ |
| IV | 8-12 " | 7 " | 263 | 0.64 " | +++ |
| V | 12-16 " | 9 " | 87 | 0.03 " | +++ |
| VI | 16-20 " | 23 " | extracts show no characteristic structure | | — |
| VII | 20-24 " | 18 " | | | — |
| VIII | 24-28 " | 15 " | | | — |
| | | | | Total 0.9 mgm. | |

* See footnote to Table VIII.

TABLE V: 28 DAY RABBIT. NEUTRAL EXTRACT OF URINE

| Fraction | Time | Wt. of extract | $E^{1\%}_{1\ cm.,\ 2970\ A.}$ | Wt. of dibenzanthracene | Agreement * with shape of theoretical curve |
|---|---|---|---|---|---|
| III | 4-8 days | 8.5 mgm. | 55 | 0.07 mgm. | ++ |

* See footnote to Table VIII.

TABLE VI: 28 DAY RABBIT. NEUTRAL EXTRACTS FROM CARCASS

| Organ, etc. | Wt. of extract (saponified) | $E^{1\%}_{1\ cm.,\ 2970\ A.}$ | Wt. of dibenzanthracene | Agreement * with shape of theoretical curve |
|---|---|---|---|---|
| Liver | 1.34 gm. | 7.6 | 1.6 mgm. | +++ |
| Gall bladder | — | — | nil | — |
| Blood | — | — | nil | — |
| Gastrointestinal tract with contents | 0.71 " | 14.6 | (1.7) " | + |
| Ethereal washings from peritoneal cavity | — | — | nil | — |
| Residual carcasses | 4.1 " | 5.0 | (3.3) " | + |
| | | | Total less than 6.6 mgm. | |

* See footnote to Table VIII.

TABLE VII: 28 DAY RABBIT. DISTRIBUTION OF DIBENZANTHRACENE AND DIHYDROXYDIBENZANTHRACENE

| | Dibenzanthracene | Dihydroxydibenzanthracene |
|---|---|---|
| Feces | 51.3 mgm. | 11.3 mgm. |
| Urine | 0.07 " | 0.9 " |
| Liver | 1.6 " | 0.0 " |
| Gastrointestinal tract and contents | (1.7) " | 0.0 " |
| Gall bladder | 0.0 " | 0.0 " |
| Blood | 0.0 " | 0.0 " |
| Ethereal washings from peritoneal cavity | 0.0 " | 0.0 " |
| Remainder of carcass | (3.3) " | 0.0 " |
| Total | 57.9 mgm. | 12.2 mgm. |
| Injected | 150 mgm. | |
| Recovered | 39% | 8.2% |
| Excreted | 34% | 8.2% |

lite (Table IV), but it is difficult to be certain how much of this was derived from contamination with feces in the metabolism cage. The concentration of the phenol in the urine varied in a parallel manner with the phenol content of the feces.

The livers of the 28 day and the 4 day rabbit contained 1.6 and 8.5 mgm. of dibenzanthracene respectively, but no trace of the phenolic metabolite was detected in either case, and no fluorescent material was extracted from the gall bladders. The nonsaponifiable fractions of the residual carcasses gave spectra in which there was hardly any evidence of the dibenzanthracene bands. The figures of 3.3 mgm. for the 28 day rabbit and 25 mgm. for the 4 day rabbit represent theoretical upper limits for the amount

TABLE VIII: 4 DAY RABBIT. DISTRIBUTION OF DIBENZANTHRACENE AND DIHYDROXYDIBENZANTHRACENE

(a) DIBENZANTHRACENE

| Fraction | Wt. of extract | $E^{1\%}_{1\ cm.,2970\ A.}$ | Wt. of dibenzanthracene | Agreement * with shape of theoretical curve |
|---|---|---|---|---|
| Feces | 0.535 gm. | 100.0 | 5.0 mgm. | ++++ |
| Urine | 1.016 " | 17.7 | 2.8 " | +++ |
| Contents of stomach and small intestine | 0.657 " | 228.0 | 23.5 " | ++++ |
| Contents of cecum and large intestine | 0.860 " | 73.0 | 11.8 " † | ++++ |
| Tissue of gastrointestinal tract | 12.223 " | 0.605 | 1.2 " | +++ |
| Washings from peritoneal cavity | 0.015 " | 27.0 | 0.06 " | +++ |
| Liver | 7.770 " | 0.70 | 8.5 " | ++ |
| Gall bladder | (No fluorescent extract) | | — | — |
| Blood | (No fluorescent extract) | | — | — |
| Residual carcass | 18.82 | 8.7 | Less than 25 mgm. | + |

(b) DIHYDROXYDIBENZANTHRACENE

| Fraction | Wt. of extract | $E^{1\%}_{1\ cm.,3050\ A.}$ | Wt. of dihydroxydibenzanthracene | Agreement * with shape of theoretical curve |
|---|---|---|---|---|
| Feces | 1.60 gm. | — | trace | — |
| Urine | 0.007 " | 53.4 | Less than 0.13 mgm. | + |
| Contents of stomach and small intestine | 0.157 " | 100.0 | 5.5 mgm. | +++ |
| Contents of cecum and large intestine | 0.106 " | 308.0 | 11.3 " | +++ |
| Tissue of gastrointestinal tract | 0.293 " | 6.7 | 0.7 " | ++ |

* The deviation from the shape of the theoretical curve is caused by general absorption of nonspecific components in the extracts. In the cases where the agreement is represented by ++++, the analytical data can be accepted as quantitative within the tolerances of Table II in the previous publication (9). Where the agreement is represented by +++, ++, or +, the analytical figures are progressively less accurate. In the case of dibenzanthracene analyses these data set an upper limit to the hydrocarbon content.

† Corrected for estimated loss of 20% during manipulation.

the rat. In the 28 day experiment 11.3 mgm. were eliminated in the feces (7.5 per cent of the injected hydrocarbon) while in the 4 day animal 16.8 mgm. were found in the contents of the intestinal tract.

The urine contained only traces of unchanged dibenzanthracene, and the higher figure (2.8 mgm.) observed in the case of the 4 day rabbit was due, in part at least, to leakage from the injection site, of some of the tricaprylin solution. The urine extracts contained appreciable amounts of the phenolic metabolite of dibenzanthracene that might be present, and the actual figures must be very much below these.

## DISCUSSION

The accuracy of the analytical data reported in this and the preceding paper varies from one extract to another, and depends on the interference caused by general absorption of secondary components of the extracts. A method was described in the first paper of this series by which the magnitude of this error

can be estimated approximately. The significance of these errors can be gauged from the column headed "Agreement with shape of theoretical curve" in Tables II to VIII. The arguments that are developed in the following discussion are based on gross differences in hydrocarbon and metabolite distribution, which are unlikely to be affected by such variations in the accuracy of the analytical technic.

The qualitative differences in the metabolism of dibenzanthracene by the rat and rabbit are accompanied by quantitative differences that may be equally

is excreted in one or other of the two known forms, although elimination in other forms is not excluded, as the recoveries do not approach 100 per cent. Whether or not other metabolites play a part in elimination of dibenzanthracene from the rabbit, the experiments described here indicate that elimination takes place much faster from the rabbit than the rat. After 4 days only small amounts of the hydrocarbon are found in the carcass of the rabbit (excluding the intestinal tract contents) while the rat carcasses after 14 days still contain large amounts of dibenzanthracene.

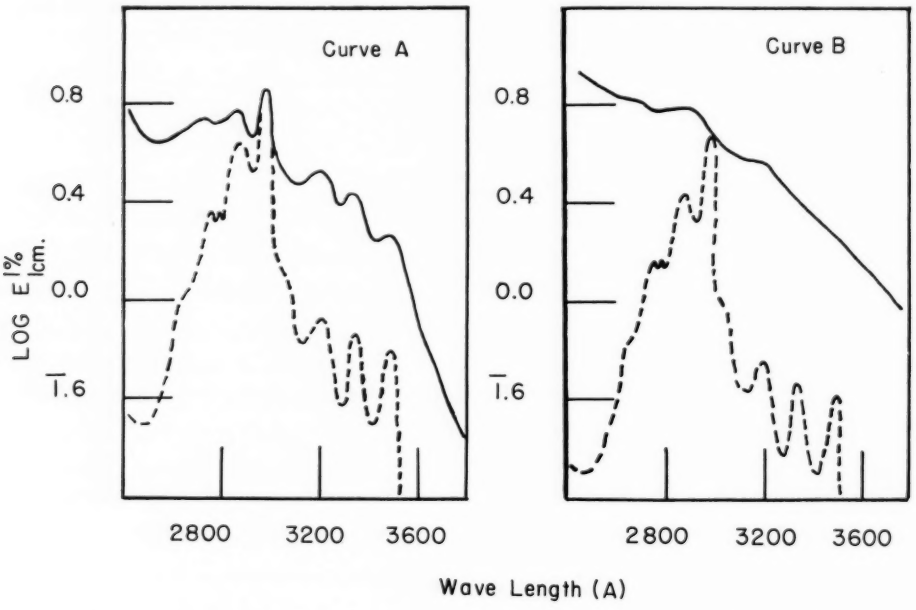


FIG. 4.—28 day rabbit. Continuous line ———— ultraviolet absorption spectra of parts of carcasses. Dash line - - - - - - theoretical curve of dibenzanthracene. (Solvent ethanol.)

Curve A Neutral fraction of liver.
" B " " " residual carcass.

significant. The results discussed in the previous section of this paper show that the rabbit is able to eliminate large quantities of the hydrocarbon with considerable rapidity, partly as the dihydric phenol and partly in the unchanged form. Forty-two per cent of the 150 mgm. injected was excreted in 28 days in one or other of these two forms, and after only 4 days 40 per cent had either been excreted or was present in the contents of the intestinal tract. The rat excreted only 7.6 per cent of injected material as dibenzanthracene and 4′,8′-dihydroxydibenzanthracene in 14 days.

Because of the low recoveries from the rat it was surmised that the major part of the injected hydrocarbon was probably metabolized to other substances, not detected by the analytical methods employed. The higher recoveries from the rabbit suggest that in this animal a much larger proportion of the material

There is considerable evidence of a qualitative nature to support the hypothesis that the main path of elimination into the intestinal tract is by way of the bile duct, and that oxidation to a phenolic derivative occurs in the liver. Chalmers (5) detected a fluorescent derivative of benzpyrene in the bile of mice that had received intravenous injections of benzpyrene. This substance is probably identical with 8-hydroxy-3,4-benzpyrene (1). Nevertheless, we have been unable as yet to detect any trace of the phenolic metabolite in the liver or the gall bladder of either animal. We have considered the possibility that the phenolic oxidation products of polynuclear aromatic hydrocarbons, which are found in feces, may be products of oxidative putrefaction in the intestine; but all attempts to obtain such derivatives by the incubation of dibenzanthracene with cultures from the intestinal tracts of rats or rabbits, under aerobic and anaerobic conditions,

have been unsuccessful, and the added hydrocarbon has been recovered quantitatively from such incubated cultures.

The significant difference in the rates of elimination from the rabbit and the rat of intraperitoneally injected tricaprylin solutions of dibenzanthracene invites speculation regarding the part that rapid elimination may play in protecting the rabbit from the carcinogenic effects of the hydrocarbon. Claude (6) has studied the continued subcutaneous injection of relatively large doses of dibenzanthracene in lard. Most of the rabbits died under 6 months, showing acute liver necrosis or degeneration and cirrhosis, but individual resistance varied, and some of the animals survived for more than 2½ years, suffering no apparent ill effects from the continued injection of the hydrocarbon. Syverton, Berry, and Dascomb (11) have recently reported that sarcomas are readily induced in cotton-tail and common domestic rabbits by the subcutaneous injection of tricaprylin solutions of methylcholanthrene, and it would be interesting to determine whether the elimination of this carcinogen from the rabbit occurs at a significantly slower rate than that of dibenzanthracene and benzpyrene.

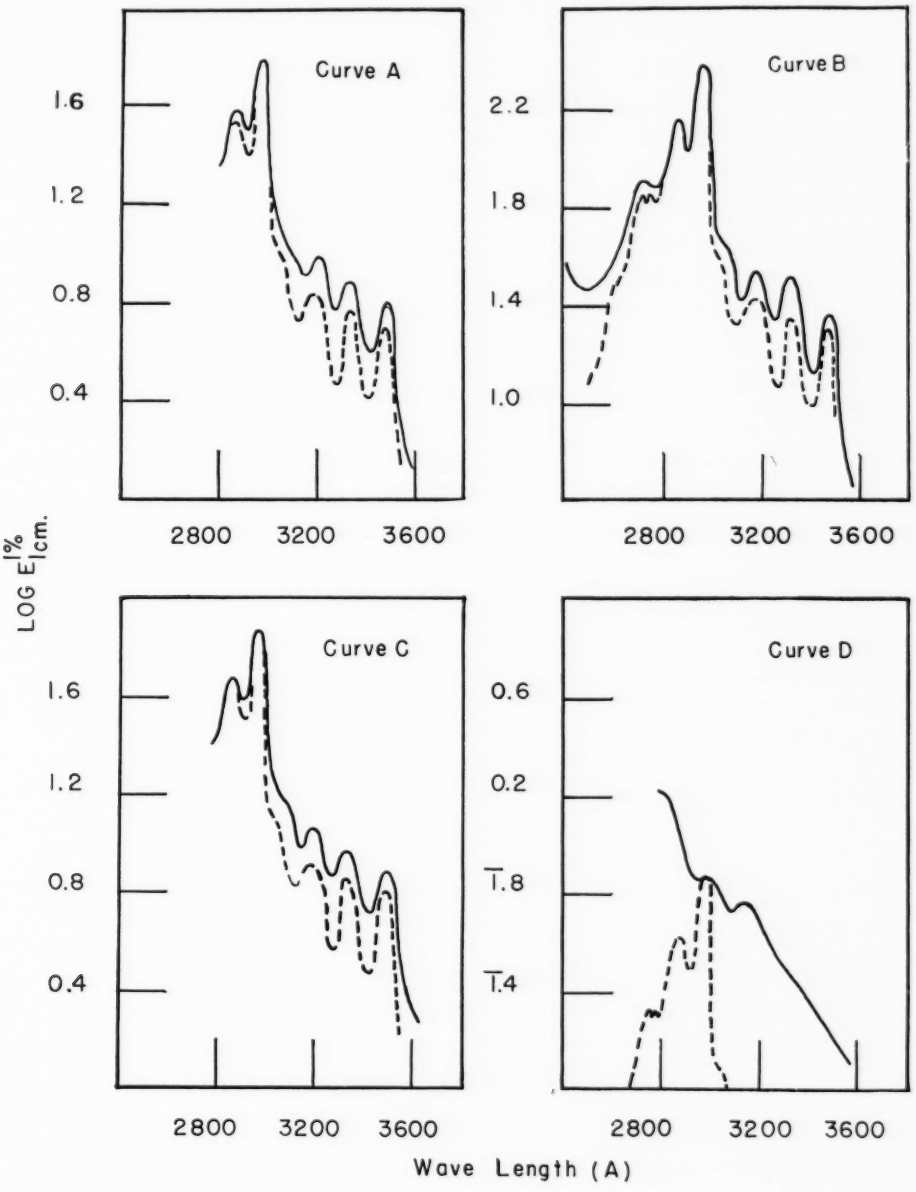


FIG. 5.—4 day rabbit. Continuous line ———— ultraviolet absorption spectra. Dash line – – – – – – theoretical curve of dibenzanthracene. (Solvent ethanol.)

| | | |
|---|---|---|
| Curve A | Neutral fraction of feces. | |
| " B | " " | Contents of stomach and small intestine. |
| " C | " " | Contents of cecum and large intestine. |
| " D | Neutral extract of liver. | |

SUMMARY

The elimination of dibenzanthracene from the rabbit has been investigated under conditions closely similar to those employed in previously reported studies of elimination from the rat.

The observation of other workers that the hydrocarbon is excreted in part as a phenolic metabolite, not identical with 4′,8′-dihydroxydibenzanthracene, has been confirmed.

The metabolism has been studied over periods of 4 and 28 days after intraperitoneal injection and it is shown that the hydrocarbon passes rapidly into the intestinal tract. About 40 per cent can be recovered from the intestinal tract and feces, either as the unchanged hydrocarbon or as the phenolic metabolite. After 16 days the rate of excretion has fallen to very low levels, and only traces of the hydrocarbon can then be detected in the carcass or excreta. The elimination is thus much more rapid from the rabbit than from the rat, and the unchanged hydrocarbon and the phenolic metabolite are probably the main forms in which it is eliminated.

The phenolic metabolite has not been detected in the liver or gall bladder, and consideration has been given to the hypothesis that the phenolic substances are produced by the action of bacteria in the intestinal tract. However, attempts to obtain such phenolic products by the incubation of dibenzanthracene with cultures from the intestinal tracts of rats or rabbits have been unsuccessful.

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REFERENCES

1. Berenblum, I., and Schoental, R. The Metabolism of 3,4-Benzpyrene in Mice and Rats. I. The Isolation of a Hydroxy and a Quinone Derivative, and a Consideration of Their Biological Significance. Cancer Research, **3**: 145-150. 1943.
2. Boyland, E., and Levi, A. A. Metabolism of Polycyclic Compounds. II. Production of Dihydroxydihydroanthraceneglycuronic Acid from Anthracene. Biochem. J., **30**:728-731. 1936.
3. Boyland, E., Levi, A. A., Mawson, E. H., and Roe, E. Metabolism of Polycyclic Compounds. IV. Production of a Dihydroxy-1:2:5:6-Dibenzanthracene from 1:2:5:6-Dibenzanthracene. Biochem. J., **35**:184-191. 1941.
4. Cason, J., and Fieser, L. F. Synthesis of 4′,8′-Dihydroxy-1,2,5,6-Dibenzanthracene and Its Relation to Products of Metabolism of the Hydrocarbon. J. Am. Chem. Soc., **62**:2681-2687. 1940.
5. Chalmers, J. G. The Elimination of 3:4-Benzpyrene and Other Polycyclic Hydrocarbons from the Mouse. Biochem. J., **32**:271-278. 1938.
6. Claude, A. Liver Degeneration and Cirrhosis Produced by 1:2:5:6-Dibenzanthracene. Am. J. Cancer, **31**:100-103. 1937.
7. Dobriner, K., Rhoads, C. P., and Lavin, G. I. Conversion of 1,2,5,6-Dibenzanthracene by Rabbits, Rats, and Mice. Significance in Carcinogenesis of this Conversion. Proc. Soc. Exper. Biol. & Med., **41**:67-69. 1939.
8. Dobriner, K., Rhoads, C. P., and Lavin, G. I. The Spectroscopic Study of Biological Extracts. II. The Detection, Isolation, and Biological Effects of the Metabolites of 1,2,5,6-Dibenzanthracene. Cancer Research, **2**:95-107. 1942.
9. Jones, R. N., Dunlap, C. E., and Gogek, C. J. The Spectrographic Analysis of Carcinogenic Hydrocarbons and Metabolites. IV. The Elimination of 1,2,5,6-Dibenzanthracene from the Rat. Cancer Research, **4**:209-217. 1944.
10. Levi, A. A., and Boyland, E. The Production of Dihydroxy-1:2:5:6-Dibenzanthracene from 1:2:5:6-Dibenzanthracene by Rabbits. Chem. and Indust., **15**:446. 1937.
11. Syverton, J. T., Berry, G. P., and Dascomb, H. E. Studies on Carcinogenesis in Rabbits. I. Malignant Tumors Induced in Cottontail Rabbits by the Injection of Methylcholanthrene in Tricaprylin. Cancer Research, **2**:436-444. 1942.

# Studies in Selective Differentiation of Tissues by Means of Filtered Ultraviolet Light *

Louis Herly, M.D.

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This study was undertaken to determine whether specificity of color can be correlated to specific tissues.

The term *Fluorescence,* as used in the observations described here, is understood to mean light phenomena that are excited by means of ultraviolet rays; therefore only such light sources could be used as are rich in emanation of ultraviolet rays and concentrate considerable intensity on a comparatively small surface. In order to observe these fluorescence phenomena, it is necessary that the ultraviolet be free or nearly free from visible light. This is accomplished by placing in front of the light source suitable filters that permit the passage of ultraviolet rays only. Such light then becomes *filtered ultraviolet light.*

Several filters are available. The "Wood Filter" consists of a gelatine film on cobalt glass, stained with sodium salt of methyl-nitraniline. In the Corning filter used in this study the coloring agent is distributed throughout the glass rather than confined to surface flashing.

Filtered ultraviolet light, as used in this work, has a wave length in the neighborhood of 3,650 A.

## APPARATUS

The ultraviolet radiations were obtained from a lamp supplied by the courtesy of Pfaltz and Bauer, as well as from a Hanovia lamp. These lamps were used interchangeably. The Pfaltz and Bauer lamp consists of an 85 watt mercury capillary arc enclosed in a protective glass bulb. The necessary voltage is supplied by a transformer connected to a 110-120 volt a.c. line. The socket that holds this lamp is adjustable vertically and the carriage in which the socket is held is adjustable horizontally. Behind the lamp is a reflector and in front a condensing lens system. An iris diaphragm permits control of the light intensity; a filter holder to accommodate the spectral filter is directly in front of the iris diaphragm. The filter permits passage of ultraviolet light (maximum intensity about 3,700 A., filtering out all significant visible rays. The filters used were "Heat Resisting Corning Red Purple Ultra No. 5874" and "Red Ultra No. 5840." All these parts are incorporated in the lamp housing. This housing is mounted on a base and is adjustable from side to side by means of a screw to permit accurate adjustment of the light beam. The Hanovia ultraviolet lamp was "Type 16200" of similar construction to the Pfaltz and Bauer lamp and was used interchangeably with the latter. The same filters were used in both lamps. Examination of specimens was made in a dark room.

For use in the operating room the Technicon Company of New York constructed a wooden box that enclosed the light source. The inside of this box was lined with asbestos that had been previously sprayed with carbon-black, as it was impossible to obtain a black dye that did not fluoresce. On two sides of this box were installed telescopic sights, through which two persons could study the specimen simultaneously. This box is of such size and shape that it can be placed conveniently on a small table in a corner of the operating room.[1]

The following is the technic employed for obtaining color photographs of ultraviolet fluorescence:

Film: Professional Daylight Kodachrome film.
Lamp: One Hanovia Type 16200 ultraviolet lamp.
Filters: U.V. #4 filter over the lamp.
86A filter over the camera lens.
Lens: 4 × 5 Bausch and Lomb Tessar, series #1C.
Exposure: 45 minutes at f6.3.
Measurements: Camera filter to specimen, 32 cm.
Lamp filter to specimen, 25 cm.
Bellows extension, 24 cm.

Photographic reproductions of the colors seen in the tissues when these were viewed in filtered ultraviolet light were prepared to illustrate the relative differences observed between benign and malignant tissues, and also for the purpose of establishing a permanent record.

* The material for these studies was obtained from the Department of Pathology of Memorial Hospital, N. Y. The clinical observations were made in the operating room of the Breast Department of Memorial Hospital, N. Y.

[1] At the present time experiments are under way with a compound lens system to be installed in this box in order to obtain magnification without distortion of the specimen.

### Previous Fluorescence Studies

Helmholtz (9) in 1896 and Hess (10) in 1911 observed fluorescence in the eyes of insects and also in the lens of the eye in vertebrates.

The foundation of luminescence phenomena was laid by Wood (1903) and Lehmann (1910), who were physicists and chemists rather than biologists. Goldstein (5) and De Kowalski (3) pointed out "the remarkable luminosity reflected by skin, muscle, the cortex of plants and certain microbes."

H. Stübel (23) studied fluorescence phenomena in animal tissues. He observed that nearly all animal cells and tissues possess the property of fluorescing in filtered ultraviolet light; he found that the presence of pigment, hemoglobin and Hb derivatives, diminishes or prevents fluorescence and that bacterial growth and necrosis change the colors observed. Structure of the tissue influences the intensity of the fluorescence. According to Stübel (23):

The *hair of the head* usually shows no fluorescence, but that of the axilla (perspiration) fluoresces orange. *Connective tissue* fluoresces intensely bluish-white. *Blood vessels, bronchial tubes, and serous membranes* show a bluish-white fluorescence, the intensity being more or less dependent on the age of the individual. The *brain membranes* are an intense bluish-white, the *brain cortex* yellow, the *white matter* faintly pinkish, and the *cerebellum* grayish-yellow. The *mucous membrane of the tongue* is grayish-white to yellowish-white; occasionally orange fluorescence is observed in the middle of the *back of the tongue. Skeletal musculature* appears dark brown to black-brown with hardly any fluorescence. *Cartilage* is bluish-white. *Thyroid gland* is reddish-gray. *Carotid gland* is intensely red. *Pancreas* has a brownish-yellow color with little fluorescent quality. *Testicle,* on the cut surface, is dark gray-red. *Liver* is yellow-green to brown-yellow.

This variation in the fluorescence of human tissues and organs prompted Havlicek (7) to hang an ultraviolet lamp over his operating table, in an attempt to make differential diagnoses of various diseases. The results were not published.

Danckwortt (2), who cites Havlicek's work, remarks that this method might become of great importance, if luminescent substances stored in malignant tissues could be brought to fluoresce in ultraviolet light. Most investigators who took up this challenge searched for dyes that would stimulate secondary radiations. Fluorescein, Trypaflavin, and Rivanol were used in these attempts. Some have injected fluorescent substances into the subcutaneous tissues, the lymphatics, and the blood vessels. Valuable observations were made in this manner in the study of liver and kidney secretions and in defining the limits of tissue death from deficient or absent blood circulation. Fluorescence–microscopy, as employed by Haitinger and Hamperl (8), has enabled these observers to follow both primary and secondary (induced) fluorescence in various cells and tissue spaces.

Fluorescent substances found in normal as well as pathological conditions have been investigated by Squires (21, 22). Studying the blood serum in health and disease, Reche (15, 16) found that in the presence of illness it fluoresced in a variety of colors whereas in health it did not.

The skin eruption of contagious diseases such as measles can be observed in ultraviolet light before it can be detected in ordinary light. Some dermatologists use ultraviolet light to differentiate between hyperkeratoses and psoriasis. Roffo (17, 18) has attempted to make an early diagnosis of skin carcinoma by means of ultraviolet light.

Observations of characteristic fluorescences have been made in lower forms of life, and a summation of the biological work in this special field may be found in Guillaume's book (6).

The differentiation of bacterial cultures in filtered ultraviolet light was first described by Arloing, Policard, and Langeron (1), and characteristic colors and intensities of various forms of bacteria were described by Gassul and Žolkevič (4).

Autofluorescence of certain tissues has been reported by a number of observers, among them Sutro and Burman (24), who though they were "unable to find a specific correlation between fluorescent reaction and pathological diagnosis" state that "a cellular tumor that has extended into the muscular layer of the stomach or rectum will fluoresce from a purple to a deep brown," and that "a medullary carcinoma in a zone of fibrous tissue appears purple against white."

The factor responsible for the fluorescence of the tissues is at present unknown. The following theories have been advanced:

1. It is due to certain pigments contained in the cells.

2. The proteins in the cells and tissues are responsible.

3. According to Roffo, the cholesterol present in the tissues fluoresces when exposed to ultraviolet light (17, 18).

4. Tissues contain fluorescent vitamins.

5. It is caused by the difference in ratio between organic and inorganic matter in the tissues. Cancer cells contain more mineral matter.

This last theory derives some support from the reports of experiences with microincineration of tissues, benign and malignant, as reported by Horning and

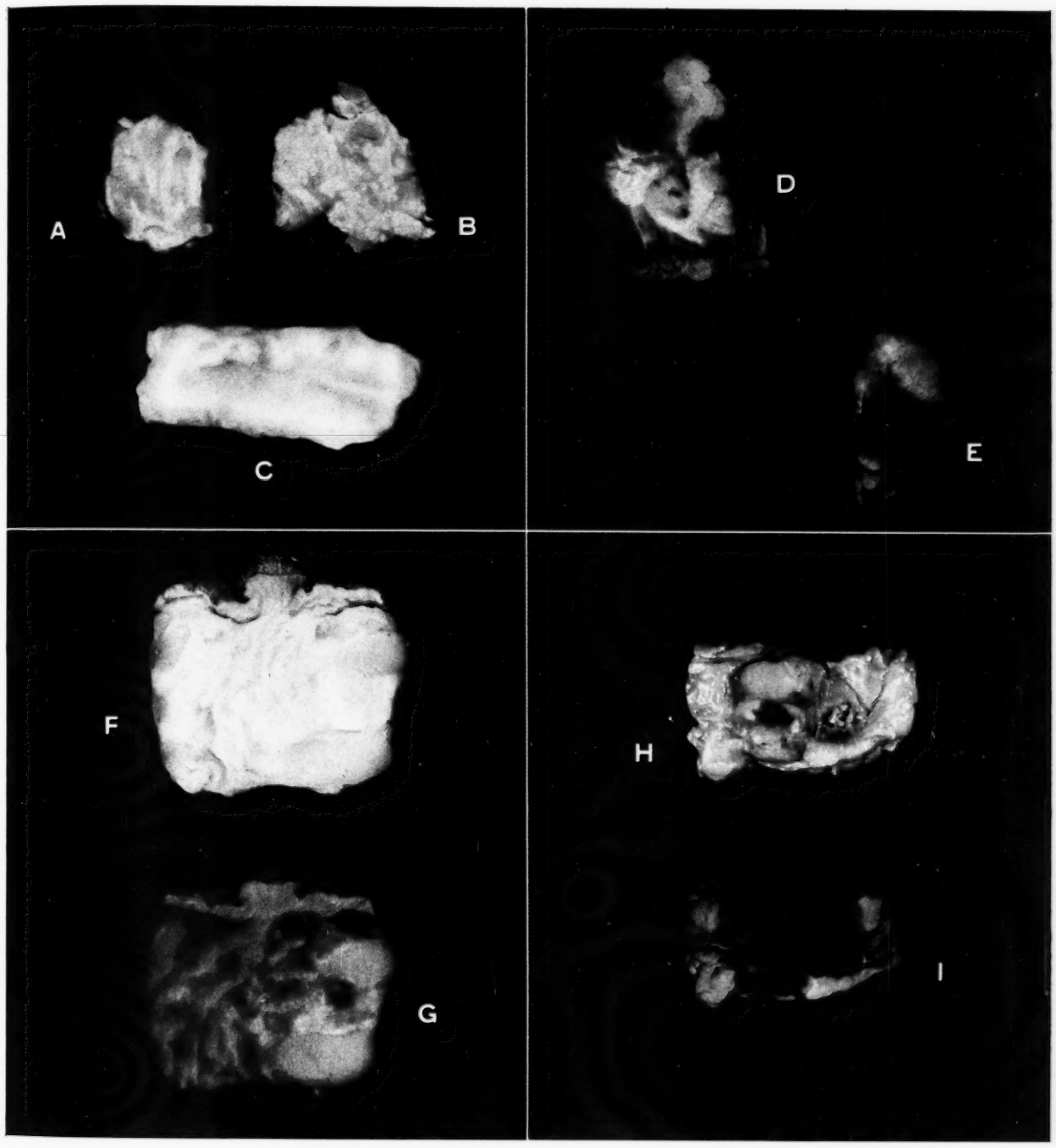



FIG. 1.—Photographs of breast tumors in filtered ultraviolet light. (A) Cystic mastitis. (B) Papillary cystadenoma of breast (C) Fibrous mastopathy. (D) Infiltrating duct carcinoma of breast, grade 2. (E) Metastatic axillary node of (D). (F) Infiltrating duct carcinoma of breast, diffuse lymphatic permeation. (Photographed in ordinary light.) (G) Same as (F) in ultraviolet light. (H) Metastatic melanoma in breast, primary tumor in thigh. (Photographed in ordinary light.) (I) Same as (H) in ultraviolet light.

Richardson (11), Hueper (12), and Policard and Doubrow (14). Scott (20) states that this technic permits a study of the distribution and localization of inorganic salts in cells and tissues. According to Scott and Horning (19), "analysis of breast carcinomas shows that the infiltrating cancerous growths are characterized by a heavy deposition of mineral salts. The nuclei of mammary cancer contain rather more ash residue than do the nuclei of cells of normal duct tissue." Olch (13) examined neoplasms of the breast and skin by the method of microincineration. "Ash obtained by microincineration of a section of *normal resting breast* is flat white, indicating (according to Policard) the presence of calcium. In *chronic cystic mastitis* marked differences were observed, corresponding in degree with the state of the duct and alveolar epithelium. When there is no evidence of proliferation of the duct epithelium, the residue is white. When in addition to cyst formation, there is evidence of epithelial proliferation, upon incineration, it leaves a decidedly bluish ash and a decreased amount of white ash. In every *carcinoma of the breast,* no matter what type, this blue color of the mineral ash of malignant epithelial cells was pronounced. The ash of the stroma in carcinoma of the breast varies from white to bluish white. Where there is epithelial degeneration the blue color decreases."

## EXPERIMENTAL

Filtered ultraviolet light excites in cells and tissues specific fluorescent spectra, a phenomenon known as autofluorescence, or primary fluorescence.

An attempt was made to determine whether different tissues fluoresce in specific colors and whether such specificity of colors can be employed in differentiating malignant from benign lesions.

The breast was selected as the first organ for study since there was a greater amount of material available and because it is in this field that the surgeon most frequently is confronted with the problem of making an immediate diagnosis to determine the extent of the operation to be undertaken.

It appeared that the primary fluorescence excited in these tissues by the filtered ultraviolet light used in this study, might be employed by the surgeon, in the operating room, in an examination of the fresh specimen, for the tissues are seen in a variety of colors, without fixation or staining. The architecture of the tissues under examination becomes sharply outlined. Small nodules, otherwise hardly visible, become clearly defined. Lymph nodes invaded by malignant cells apparently duplicate the fluorescent features of the primary tumor.

The attempt was made to differentiate between benign and malignant lesions by an application of these color phenomena. All varieties of diseases and neoplasms of the breast were examined for their fluorescent properties; certain variations were found, all of which could be correlated with organ structure.

Over 200 of these examinations in filtered ultraviolet light were made in the operating room immediately after removal. The diagnoses have been fairly consistently correct. They were checked against the microscopic diagnoses of the pathologist. One error was made with the method as against one made by the frozen section method. A permanent microscopic section was made of the surface of every specimen studied to corroborate the diagnostic colorings as seen and reproduced in the photographs.

The colors [2] observed in the fluorescing tissues were roughly as follows:

White (bright): normal breast tissue; scar tissue.
Blue (blue-white): connective tissue. Blood vessels, nerves, and skin on cross section.
Blue (cobalt, in various tones): fibroadenoma, cystic mastitis (small cysts).
Blue (Dresden with coarse white lines): sclerosing adenoma.
Green (olive): slight remains of absorbing hematoma.
Pink (dull): necrosis.
Red (geranium, in spots): bacterial invasion.
Red edges: superficial carcinomatous ulceration.
Black (or very dark brown): blood; blood clot.
Orchid (faint and dull): lining of cyst wall.
Purple (varying in intensity and frequently with fine, chalky, bright white lines): carcinoma; where the cancer cells are tightly packed and where nuclei are especially hypertrophic and hyperchromatic, the purple color passes into tan, orange, and brown.

The possibility that changes in the fluorescent colors might be a result of variations in the pH of the tissues has been tested by changing their pH. No changes were observed.

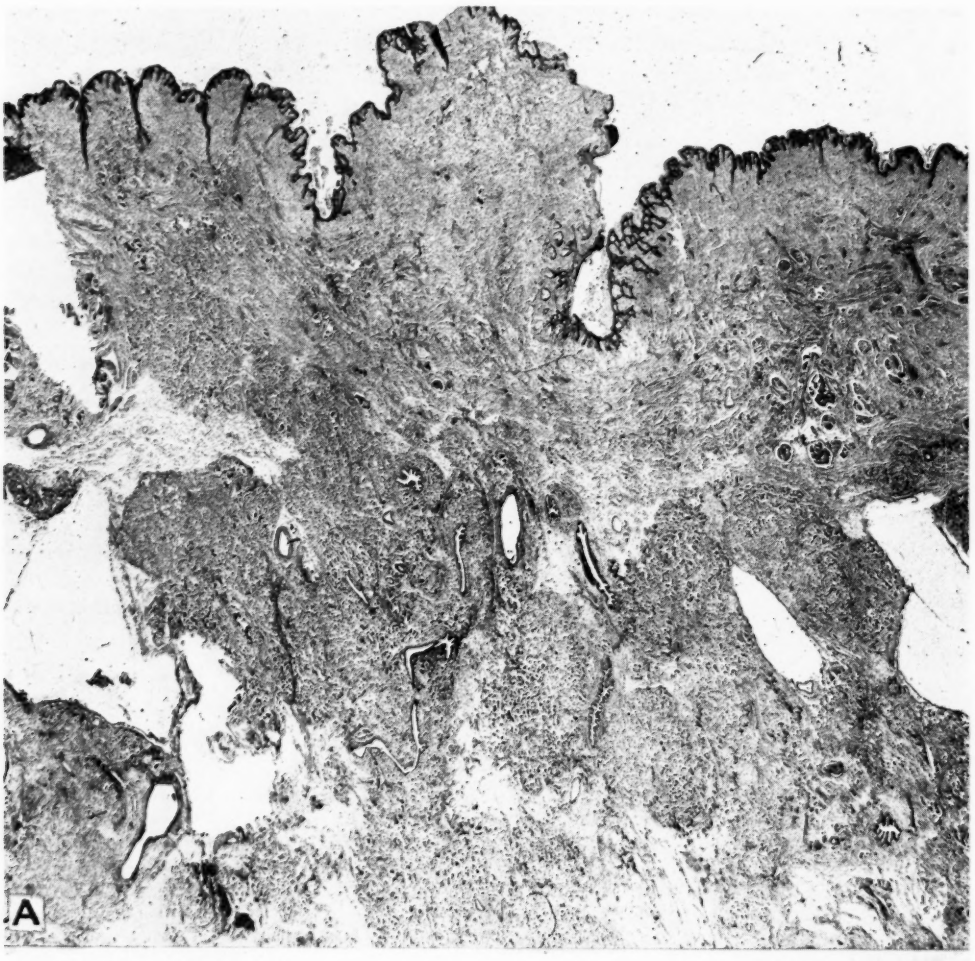
Observations of emission spectra from the tissues were correlated with the colors in the photographs. They seemed to be within the same spectroscopic range. A hand spectroscope (Hellige) was used and the wave lengths of the various bands were found to range between 3,600 and 4,000 A.

Rapidly growing tissues such as embryos and regenerating liver were tested for emission spectra. These tissues, it was found, had no primary fluorescence.

The method was tested on experimental animal tumors also. No specific colors such as were observed in human tumors were detected. The reason for this variation is at present obscure and requires further investigation.

The photographic reproductions of the colors observed in tissues under filtered ultraviolet light have not so far been true reproductions of the colors seen by the eye. Very faint blue-white is much darker in

[2] The description of the colors is based on the color chart in Webster's New International Dictionary, 1942, p. 528.



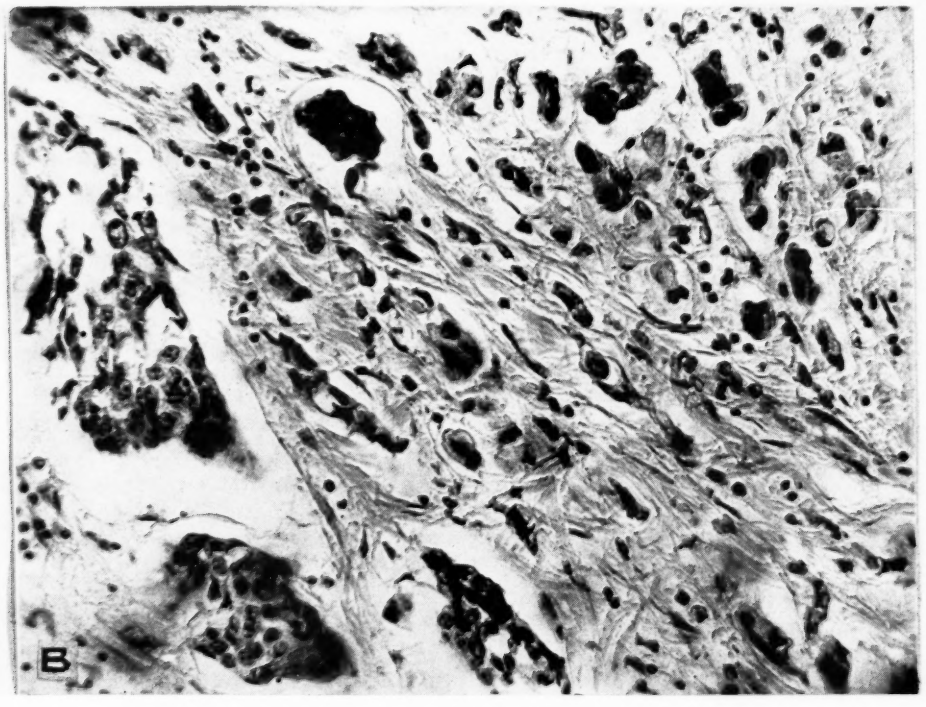




FIG. 2.—Microphotographs of F and G (Plate I). A. Low power. B. High power.

the photographs; shadows, depressions, and cavities reproduce dark blue, whereas the eye sees them in lighter and more grayish hues. We have also been unsuccessful in reproducing the various tones of purple. These deficiencies should be taken into consideration in studying the photographs.

To obtain a truer reproduction of colors it will be necessary to construct a highly sensitive photometer, as the primary fluorescence of the tissues is very low in light intensity.

## SUMMARY AND CONCLUSIONS

1. Normal breast as well as many forms of breast disease, inflammatory and neoplastic, was studied in filtered ultraviolet light.

2. An attempt was made by means of the primary fluorescence evoked in tissues by filtered ultraviolet light to differentiate benign lesions from malignant disease of the breast. Over 200 specimens of pathological breast tissue were examined immediately after removal in the operating room. One error was made with the method as against one made with the frozen section method.

3. Characteristic specimens of various types of breast lesions were studied and photographed in filtered ultraviolet light.

4. Permanent microscopic sections were made of the tissue surfaces photographed to corroborate the diagnosis based on the fluorescent colors.

630 West 168th St.
New York 32, N. Y.

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## BIBLIOGRAPHY

1. Arloing, F., Policard, A., and Langeron, L. Aspects offerts par diverses cultures microbiennes sous l'action des Radiations émises par la lampe a arc au mercure après filtration par des écrans de Wood (Lumière de Wood). Compt. rend. Soc. de biol., **92**:261-263. 1925.
2. Danckwortt, P. W. Luminiszenz-analyse im Filtrierten Ultravioletten Licht. Leipzig: Akademische Verlagsgesellschaft M. B. H. 1934.
3. De Kowalski, J. Quelque phénomènes de fluorescence avec expériences de Kowalski. Cited by Danckwortt.
4. Gassul, R., and Žolkevič, A. Ueber Differenzierungsversuche an Bakterienkulturen mit Hilfe der sogenannten Woodschen Strahlen. Vorläufige Mitteilung, Centralbl. f. Bakteriol. (Abt. 1), **104**:503-507. 1927.
5. Goldstein, E. Ueber die Untersuchung der Emissionsspektra fester aromatischer Substanzen mit dem Ultraviolet Filter. Cited by Danckwortt.
6. Guillaume, A. C. Les Radiations Lumineuses en Physiologie et en Thérapeutique. De l'infra-rouge à l'ultraviolet. Paris: Masson et Cie. 1927.
7. Havlicek, H. Die Verwendung des Woodschen Lichtes zur Diagnose und Schmerzbetäubung bei Operationen. Cited by Danckwortt.
8. Haitinger, M. J., and Hamperl, H. Die Anwendung des Fluoreszenzmikroskops zur Untersuchung tierischer Gewebe. Ztschr. mikr.-anat. Forsch., **33**:193-221. 1933.
9. Helmholtz. Physiologische Optik, 2 Auflage, pp. 284-306, 1896.
10. Hess, C. Ueber Fluorescenz an den Augen von Insekten und Krebsen. Pflüger's Arch. f. d. ges. Physiol., **137**: 339-349. 1911.
11. Horning, E. S., and Richardson, K. C. Cytological Differences between Normal and Malignant Tissues. M. J. Australia, **1**:238-247. 1930.
12. Hueper, W. C. Histochemical Studies of Organs of Tumor-Bearing Rats by the Microincineration Method. J. Lab. & Clin. Med., **19**:1293-1303. 1934.
13. Olch, I. Y. The Examination of Neoplasms of the Breast and Skin by the Method of Micro-Incineration. Proc. Soc. Exper. Biol. & Med., **30**:511-513. 1933.
14. Policard, A., and Doubrow, S. Récherches Histochimiques sur la Teneur en cendres des cancers. Ann. d'anat. path., **1**:163-171. 1924.
15. Reche, O. Fluoreszenzdiagnose. Münch. med. Wchnschr., **78**:1591-1593. 1931.
16. Reche, O. Fluoreszenzdiagnostik. Münch. med. Wchnschr., **78**:2051. 1931.
17. Roffo, A. H., and Roffo, A. E., Jr. La fluorescencia de las lesiones hipercolesterínicas precancerosas, excitada con la luz de Wood. Prensa méd. argent., **26**:2511-2528. 1939; also Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **16**:209-253. 1939.
18. Roffo, A. H., and Roffo, A. E., Jr. La fluorescencia de las lesiones hipercolesterínicas de la piel en el diagnóstico precoz del cáncer; método de fotografía directa. Bol. Inst. de med. exper. para el estud. y trat. d. cáncer, **18**:69-115. 1941; also Prensa méd. argent., **28**:1247-1257. 1941.
19. Scott, G. H., and Horning, E. S. Histochemical Studies by Microincineration of Normal and Neoplastic Tissues. Amer. J. Path., **8**:329-332. 1932.
20. Scott, G. H. The Distribution of Inorganic Salts in Adult and Embryonic Cells and Tissues. The Cancer Problem. New York: Science Press. 1937, pp. 173-179.
21. Squires, B. T. The Ultra-Violet Fluorescence of Urine. Proceedings of the Physiological Society, October 13, 1928. J. Physiol., **66**:xiv-xv. 1928.
22. Squires, B. T. Cited by Danckwortt.
23. Stübel, H. Die Fluoreszenz tierischer Gewebe in ultravioletem Licht. Pflüger's Arch. f. d. ges. Physiol., **142**: 1-14. 1911.
24. Sutro, C. J., and Burman, M. S. Examination of Pathologic Tissues by Filtered Ultraviolet Radiation. Arch. Path., **16**:346-349. 1933.

# Chromosome Complexity in Regenerating Rat Liver

John J. Biesele, Ph.D.*

*(From the Department of Zoology, the University of Pennsylvania, Philadelphia, Pa.**)*

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Because the rapid growth of regenerating rat liver is suggestive of that in some malignant tumors, it is of interest to determine whether chromosome size differences such as those found to exist between normal tissues and cancers of man (4), mouse (5), and goldfish (2) are present between control and regenerating livers. Embryonic tissue likewise grows rapidly, but at least in the fetal liver of the mouse no enlarged chromosomes are to be seen (5). It is conceivable that noncancerous adult tissues of vertebrates do contain some polytene chromosomes but that they are masked by failure of the cells containing them to divide, as Clara (8) would have supposed, since such cells could be assumed to be too highly differentiated to undergo mitosis in the day-by-day replacement of cells in the adult organ. The occasional dividing cells would then be some of those that have retained chromosomes of embryonic character. Polytene chromosomes could be thought of as coming to light in cancerous tissues because of a very great drive to cell division inherent in such tissues. The stimulus to growth that removal of most of the liver provides to the remaining lobe might be supposed to be drastic enough to call forth mitotic activity in all cells of the lobe. It is not certain that this last is true, of course.

In summary, the search for chromosome changes from control to regenerating liver was made in the hope of establishing or disproving either that some condition involved in rapidity of growth produces enlarged chromosomes at the time of growth, or that enlarged, polytene chromosomes already present but hidden in some tissues may be called forth into view in the intense mitotic activity of regeneration. Since no chromosomal changes were found in the regenerating liver, we must look elsewhere for the reason for polytene chromosomes in cancers.

## MATERIAL AND METHODS

Four male white rats of the same litter, about 3 or 4 months old and in good health, were subjected to partial removal of the liver, as described by Higgins and Anderson (11). As control, some of each excised liver was preserved in Carnoy's acetic-alcohol fixative and later stained in acetocarmine, pressed out on slides, dehydrated in alcohol, and mounted in diaphane. On the second, fourth, sixth, and eighth days after operation the animals were sacrificed, bits of the regenerating livers were fixed in Carnoy's fluid, and slides were prepared as in the case of the control tissues.

In the microscopic study of each tissue as many suitable metaphase (or, by mistake, late prophase) figures as could be found, up to 25, were drawn with the aid of a camera lucida and the use of oil immersion, a pair of 20× ocular lenses, and a table magnification of 3,000 times. For a given figure, certain of the chromosomes were found oriented with the plane determined by the axes of the two chromatids parallel to the focal plane. The width of such chromosomes was measured with a millimeter ruler under the mirror of the camera lucida, and one-fourth of that width was then used as the chromatid radius in determining the volumes of all the individual chromosomes. The length of each chromosome was measured to the nearest one-third millimeter, *i.e.,* about one-tenth micron, on the drawing, and its volume was then computed as the product of 2, Pi, the square of the chromatid radius, and the length, to the nearest one-tenth cubic micron. An average chromosome volume was calculated for each figure in practice by determining the average chromosome length and multiplying it by the product of the other factors, which are constant for a given figure.

## RESULTS

A summary of the data appears in Tables I and II.

It is evident that there was no consistent difference between the control and the regenerating livers with respect to average chromosome volumes, since an average of about one cubic micron was maintained throughout. Moreover, the frequency distributions of metaphases with average chromosome volumes ranging from 0.6 to 1.7 cubic microns were quite similar for the regenerating livers and their controls (Table II). These are hardly normal unimodal distributions, however; rather, they are better interpreted as bimodal, for both control and regenerating liver. The averages

* Fellow of The International Cancer Research Foundation.

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of the values under the two peaks are 0.8 and 1.2 cubic microns. The ratio that these values bear to one another, 2:3, is not that expected for simple and double chromosomes, 1:2.

The last three columns of Table I give the percentages of the figures drawn of diploid, tetraploid, and octoploid chromosome numbers respectively. The aneuploid figures are also included here, for the upper limit of a given ploidy was taken as one and one-half times the chromosome number of that ploidy. It will be recalled that the diploid chromosome number of the rat is 42 (12). Hence the upper limits of the diploid, tetraploid, and octoploid ranges were taken as 63, 126, and 252 chromosomes respectively. No consistent trend in change of proportions of figures of the various ploidies with time after operation is discernible. In fact, the variation among the four control tissues seems to indicate that the samples were too small to allow any definite conclusions on this matter. In the overall view, 54.5 per cent of the control metaphases and 59 per cent of the regenerating liver metaphases had chromosome numbers in the diploid range; 40.5 per cent of the control and 37 per cent of the regenerating liver metaphases were in the tetraploid range, while 5 and 4 per cent of the metaphases of the two tissues were approximately octoploid. Of the 67 metaphases with 64 or more chromosomes, *i.e.*, presumably polyploid, 30 had chromosomes that averaged less than 1 cubic micron, and 37 had chromosomes that averaged more than 1 cubic micron. This ratio of 30:37 corresponds fairly well to the 76:86 ratio of all the metaphases with respect to chromosome volumes under and above 1 cubic micron respectively. Hence we can show no association between polyploidy and enlarged chromosomes.

TABLE I: AVERAGE CHROMOSOME VOLUMES AND APPROXIMATE PLOIDIES OF THE METAPHASE FIGURES IN REGENERATING AND CONTROL LIVERS OF THE RAT

| | | Average chromosome volumes | | Metaphase figures | | | |
|---|---|---|---|---|---|---|---|
| Day | Tissue | Mean | Range | Total | Diploid, % | Tetraploid, % | Octoploid, % |
| 2nd | Control | 1.1 $\mu^3$ | .7–1.5 $\mu^3$ | 7 | 57 | 43 | 0 |
| | Regen. | 0.9 | .6–1.4 | 26 | 46 | 46 | 8 |
| 4th | Control | 1.0 | .6–1.5 | 25 | 60 | 32 | 8 |
| | Regen. | 1.2 | .9–1.6 | 25 | 76 | 24 | 0 |
| 6th | Control | 1.2 | .7–1.7 | 25 | 40 | 56 | 4 |
| | Regen. | 1.0 | .7–1.6 | 16 | 62 | 38 | 0 |
| 8th | Control | 1.0 | .7–1.4 | 13 | 61 | 31 | 8 |
| | Regen. | 0.9 | .7–1.2 | 25 | 52 | 40 | 8 |
| | | Average | Total | Total | Average | Average | Average |
| | Control | 1.1 | .6–1.7 | 70 | 54.5 | 40.5 | 5 |
| | Regen. | 1.0 | .6–1.6 | 92 | 59.0 | 37.0 | 4 |

TABLE II: FREQUENCY DISTRIBUTIONS OF METAPHASES OF VARIOUS AVERAGE CHROMOSOME VOLUMES, IRRESPECTIVE OF PLOIDY, IN CONTROL AND REGENERATING LIVERS

| Average chromosome volume | Number of metaphase figures | | |
|---|---|---|---|
| | Control | Regenerating | Total |
| 0.5–0.6 $\mu^3$ | 1 | | 1 |
| 0.6–0.7 | 2 | 5 | 7 |
| 0.7–0.8 | 13 | 12 | 25 |
| 0.8–0.9 | 8 | 17 | 25 |
| 0.9–1.0 | 8 | 10 | 18 |
| 1.0–1.1 | 7 | 16 | 23 |
| 1.1–1.2 | 10 | 15 | 25 |
| 1.2–1.3 | 5 | 6 | 11 |
| 1.3–1.4 | 6 | 4 | 10 |
| 1.4–1.5 | 3 | 2 | 5 |
| 1.5–1.6 | 5 | 4 | 9 |
| 1.6–1.7 | 2 | 1 | 3 |
| | 70 | 92 | 162 |

## DISCUSSION

The chromosomes of regenerating and control adult livers are essentially alike. They show the same volume range and the same two peaks of average volume frequency. No new complexity not present in chromosomes of the control liver arises in those of the regenerating liver. The time allowed, up to 8 days, is probably sufficient for the appearance of polytene chromosomes if any were to be formed, since the application of methylcholanthrene in benzene to the skin of the mouse has been found to be followed within 3 days by the presence of polytene chromosomes in 13 per cent of the metaphases (3), long before a maximal mitotic rate is established (9). In the regenerating liver Brues and Marble (7) have found the mitotic rate to diminish after the first 24 hours, in general. Hence we must conclude that regeneration or rapidity of mitotic growth are probably not of themselves sufficient to produce polytene chromosomes in a tissue in which none has been evident before.

The rat liver presents a more difficult subject for study of the chromosomes than do mouse tissues. The metaphases are poorly defined in rat liver, and they are not readily distinguishable from late prophases. This

fact may have contributed some of the larger average volumes to the data. Very early anaphases in some cases, and half anaphase figures, separated from their sister figures in preparation, may have been mistaken occasionally for metaphases and may have led to some of the smaller calculated average chromosome volumes. The chromosomes of the rat are more readily distorted by smearing; they are longer than those of the mouse and their consequently greater degree of overlapping causes a more confused picture. These several reasons may help explain the considerable range of variation in chromosome size encountered.

An additional cause of the range in chromosome size may lie in the different cell types of the liver. This is more likely because the frequency distribution of average chromosome volumes is bimodal, rather than unimodal as would be expected if the range in size were due solely to random error. It is probable that the bimodality stems from a difference in chromosome size between hepatic cells proper and bile duct cells. To the former we would tentatively ascribe the large chromosomes, which average 1.2 cubic microns; to the latter (perhaps along with endothelial cells) the small chromosomes, which average 0.8 cubic micron, could be assigned, in conformity with the observation of Brues and Marble (7) that the smaller bile duct cells contain mitotic figures of a smaller size than do the hepatic cells. However, it is not always true that the polyploid mitoses, which are especially prevalent in hepatic cells (1), contain the larger sort of chromosomes.

It may help to explain the situation if we consider the cytological picture in livers of newborn rats (unpublished data). There the average chromosome volume in hepatic cells is between 0.7 and 0.8 cubic micron. Diploid hepatic nuclei are about doubled in volume from the newborn to the adult rat, and the same maximum plasmosome number—6—is maintained. Because there is no increase in plasmosome number with this initial doubling in nuclear volume, I hesitate to ascribe a polytene condition to the chromosomes in adult liver. The nuclear hypertrophy seems to have occurred without an increase in the number of distinct strands in the chromosomes. However, the metaphase chromosomes of the hepatic cells have apparently increased in size about one-half in the passage from the newborn to the adult rat. Either this reflects a true hypertrophy of the chromosomes, *i.e.,* an increase in the number of molecules in each chromosome strand, quite apart from an increase in the number of strands, or it is the reflection of an increased amount of cytoplasmic substances, *e.g.,* nucleic acids or simpler nucleotides, available to attach to the chromosomes during the mitotic cycle. It is a common observation that cytoplasmic basophilia is lessened in cells undergoing mitosis, concomitantly with an increased affinity of the chromosomes for basic dyes. A doubling in cellular volume accompanying the doubling in nuclear volume, but without an increase in fundamental chromosomal material, would provide relatively more of such cytoplasmic materials for attachment to the mitotic chromosomes. Moreover, the active adult liver, noted for its vitamin content and enzymatic activity, would be expected to have a high concentration of the hypothetical nucleotides that participate in such a process. It may not be without significance that the regenerating and control adult rat livers, while agreeing with each other in their chromosomal constitution, differ from the newborn rat liver in that respect, for its chromosomes are considerably smaller, and that the same holds approximately true for the enzymatic pictures in regenerating and control adult livers as against embryonic liver (10). In conclusion, let us say that the large chromosomes of adult rat liver are probably not polytene, because their volume increases over the liver chromosomes of the very young rat is one-half rather than unity, and because there has been no increase in plasmosome number in the enlarged diploid resting nucleus.

In the dividing cells the relative proportions of diploids, tetraploids, and octoploids by actual chromosome counts showed little variation from normal to regenerating adult liver. This does not agree well with the findings of others, based on statistical studies of the diameters of resting nuclei in sectioned material. Sulkin (13) found only 10 per cent of the normal liver nuclei to have a volume that he considered indicative of diploidy, and this percentage was decreased in regenerating liver, while large nuclei (polyploids) increased in frequency. Beams and King (1) stated that the number of polyploid nuclei was increased in regenerating liver by fusion of the mitotic figures of binucleate cells, although the proportion of diploid cells remained fairly constant. Perhaps our data do not show an increase in polyploid division figures in regenerating liver because of failure of the new polyploid cells to divide as frequently as the diploid.

Light is thrown on this problem by the work of Brues and others (6, 7) on regenerating liver. There was a great amount of tissue growth the first day of regeneration, but the restoration of cell numbers lagged about one day behind tissue restoration. The material added in the initial growth phase was probably chiefly fat. Certainly very little of it was protein, since the nitrogen content of the material added the first day was only 1.4 per cent. We may consider the possibility that some of the increase in nuclear volume noted by Sulkin (13) and Beams and King (1) is a result of increased fat content and not necessarily of increased nucleoprotein content. Such a conclusion would agree

with the findings here reported of no increase in size of chromosomes and of no increase in frequency of polyploid division figures in the course of regeneration.

SUMMARY

No new chromosomal complexity not present in the control tissues arises in regenerating adult rat liver. Hence the appearance of polytene chromosomes in cancers can not be a result solely of rapid growth in adult tissues. The control and regenerating livers agreed in the proportions of diploid, tetraploid, and octoploid metaphase figures. The enlarged chromosomes of adult rat liver are held to be simple chromosomes, not polytene, because they are only about 50 per cent greater in volume than liver chromosomes of the newborn rat, and because the enlarged diploid resting nucleus in adult liver does not have an increased number of plasmosomes.

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REFERENCES

1. Beams, H. W., and King, R. L. The Origin of Binucleate and Large Mononucleate Cells in the Liver of the Rat. Anat. Rec., **83**:281-297. 1942.
2. Biesele, J. J. Diplochromosomes in a Goldfish Tumor. Cancer Research, **3**:411-412. 1943.
3. Biesele, J. J., and Cowdry, E. V. Chromosomal Changes in Epidermal Carcinogenesis. J. Nat. Cancer Inst., in press. 1944.
4. Biesele, J. J., and Poyner, H. Polytene Chromosomes in Two Mammary Carcinomas of the Human Subject. Cancer Research, **3**:779-783. 1943.
5. Biesele, J. J., Poyner, H., and Painter, T. S. Nuclear Phenomena in Mouse Cancers. Austin: University of Texas Publication No. 4243. 1942.
6. Brues, A. M., Drury, D. R., and Brues, M. C. A Quantitative Study of Cell Growth in Regenerating Liver. Arch. Path., **22**:658-673. 1936.
7. Brues, A. M., and Marble, B. B. An Analysis of Mitosis in Liver Restoration. J. Exper. Med., **65**:15-27. 1936.
8. Clara, M. Untersuchungen an der menschlichen Leber. II. Teil. Über die Kerngrössen in den Leberzellen. Zugleich über Amitosen und über das Wachstum der "stabilen Elemente." Ztschr. f. mikr.-anat. Forsch., **22**: 145-219. 1930.
9. Cooper, Z. K., and Reller, H. C. Mitotic Frequency in Methylcholanthrene Epidermal Carcinogenesis in Mice. J. Nat. Cancer Inst., **2**:335-344. 1942.
10. Greenstein, J. P. Tumor Enzymology. J. Nat. Cancer Inst., **3**:419-447. 1943.
11. Higgins, G. M., and Anderson, R. M. Experimental Pathology of the Liver. I. Restoration of the Liver of the White Rat Following Partial Surgical Removal. Arch. Path., **12**:186-202. 1931.
12. Painter, T. S. A Comparison of the Chromosomes of the Rat and Mouse with Reference to the Question of Chromosome Homology in Mammals. Genetics, **13**:180-189. 1928.
13. Sulkin, N. M. Cytological Studies of Hyperplastic Livers in the Rat. Abstract, Anat. Rec., **84**:503. 1942.

# Mitotic Incidence in the First 48 Hours of Methylcholanthrene Epidermal Carcinogenesis

Helen C. Reller, M.D., and Zola K. Cooper, Ph.D.

*(From the Department of Research, The Barnard Free Skin and Cancer Hospital, St. Louis, Missouri)*

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The present study is one of a group of investigations, organized by Dr. E. V. Cowdry and carried out by the staff of the Barnard Hospital, on the manner in which methylcholanthrene produces its carcinogenic effect on the epidermis. The study of cell division in the skin in the period during which carcinoma is developing was one of the first problems to be investigated, and the results have been reported in an earlier paper (2). Counts of the number of mitoses occurring in the epidermis were made at weekly intervals, beginning at 2 days after the first painting with methylcholanthrene and extending for a period of 13 weeks, at which time all the surviving animals showed definite carcinomas. It was found that there was an initial pronounced rise in the number of mitoses present during the first 3 weeks of the experiment. From the third to the ninth week the incidence of mitoses decreased sharply, but was still well above the average number found in untreated epidermis. From the ninth to the 13th week, when carcinoma was present in all the surviving animals in the series, there was a definite increase in the number of mitoses. After the tenth week the number of mitotic figures present vastly exceeded the numbers found in the initial rise, which occurred during the first 3 weeks of the experiment.

Although this study gave an indication of the rate of mitotic activity in the epidermis from 2 days after initial painting to the development of carcinoma, it has become increasingly evident that significant changes may occur in the first 48 hours following a single application of methylcholanthrene. Thus Mider and Morton (5) found that a single application of this carcinogen was sufficient to produce a malignant growth in the C57 brown strain of mice. Cramer and Stowell (4) and Simpson and Cramer (8) were able to confirm this finding in the Swiss and New Buffalo strains. Consequently, even a small amount of methylcholanthrene must have a profound influence upon epidermal cells. Cramer and Stowell (3) reported that during the first 2 days after a single application the epidermis of the mouse becomes transformed from 2 rows of epithelial cells into an epidermis consisting of several layers, in which basal cells, spinous cells, and cells of the stratum granulosum and stratum corneum are recognizable. In their opinion this is due to cell differentiation rather than to cell multiplication, since increase in cell number is not accompanied by a proportional increase in mitotic activity.

Other investigators, Wolbach (9, 10) and Page (6), have referred to an initial toxic and destructive effect of a carcinogen on the epidermis. Pullinger (7), who has studied the earliest changes in mouse skin after a single application of methylcholanthrene, reported an increase in the number of cells with evidence of differentiation.

In these studies, however, no actual counts of mitotic figures were made. If a changing ability of cells to divide is an expression of reaction to a toxic substance, an investigation of mitotic frequency in the epidermis immediately following the application of methylcholanthrene should give some indications as to its mode of action. The present study was undertaken to determine the immediate effect of methylcholanthrene on cell division.

## MATERIALS AND METHODS

The epidermis of the mouse's ear was chosen as the tissue for this study because whole-mount preparations can be readily made. The epidermis can be separated from the dermis completely after placing the whole skin in 1 per cent aqueous acetic acid for 24 hours in the ice box. This method has been explained in detail in earlier papers from this laboratory (Cooper and Franklin, 1, and Cooper and Reller, 2).

The conditions of the present experiment duplicate as closely as possible those of an earlier study in which mitotic counts were made on total mounts of the epidermis for the first 13 weeks of methylcholanthrene painting (Cooper and Reller, 2).

Twenty-four Strain A male mice, 2½ months of age, were kept in a room in which the temperature was maintained at 78° F. by thermostatic control, and they were fed Rockland Farms mouse pellets. None died during the experiment.

Both ears of each mouse were painted at 12 noon with 0.6 per cent methylcholanthrene (Eastman Kodak Company) in benzene, applied with a single stroke of a No. 3 camel's-hair brush to the inside

of the ear. At the same time ears were removed for comparison from 3 normal mice. At 6 hour intervals for the next 48 hours ears were removed from each of 3 mice painted with methylcholanthrene. The right ear of each mouse was placed immediately in 1 per cent aqueous acetic acid for the preparation of a total mount and the left ear in Bouin's fluid for paraffin sections to serve as checks on the degree of change.

Total mounts of the epidermis were examined at a magnification of 1,350 diameters. Nuclei were counted instead of cells as the boundaries of cells are not easily determined. The counts were made with the guidance of a ruled square inserted in the ocular. Fifteen thousand nuclei (nondividing and dividing) were enumerated in the epidermis of the inner surface of each ear. The counts were spaced so that nuclei from representative regions were included. All the nuclei that appeared in one square (usually about 55) were counted. Contiguous squares were then examined until 1,000 nuclei had been enumerated. On an average this required 18 to 20 oil immersion fields. An area equivalent to that of 1,000 nuclei was then skipped and a second group of 1,000 nuclei was counted. This procedure was repeated until the entire epithelial surface of the ear had been systematically traversed from side to side and from tip to base. Counts were not made in the necks of the hair follicles but only in the epidermis between them. For the sake of uniformity all counts were made by the same person (H.C.R.).

Paraffin sections were prepared through the medial longitudinal axis of the left ear and stained with hematoxylin and eosin. These sections served as a check on the changes in the total mounts.

Total mounts were prepared from the 3 right ears of 3 untreated Strain A male mice as mentioned above. Counts were made on each of these ears by the same method as that used on the ears of the mice that had been painted with methylcholanthrene. Thus a figure was arrived at for the normal incidence of mitosis in the epidermis of Strain A male mice.

## RESULTS

### Mitosis Counts on Total Mounts of Epidermis of Right Ears

In order to determine the normal rate of cell division in the epidermis of the ears of Strain A male mice the number of mitoses per 15,000 nuclei was counted on the right ear of each of 3 untreated animals. These counts were 9, 19, and 23 mitoses per 15,000 nuclei, an average of 17 (Table I). This figure corresponds exactly with that obtained on 5 untreated Strain A male mice in a previous experiment (Cooper and Reller, 2). These figures also correspond fairly well with those obtained on untreated mice of the Old Buffalo Strain (Cooper and Franklin, 1). In those experiments similar counts were made upon the epidermis of the ears of 24 male mice of exactly the same age, from which the ears were removed at hourly intervals for 24 consecutive hours. The average of those 24 counts was 21.7 mitoses per 15,000 nuclei.

In the present experimental group of mice the ears were removed from the first 3 individuals 6 hours following the first painting with methylcholanthrene. The mitotic counts on these ears were 7, 22, and 11 mitoses per 15,000 nuclei (Table II). The average number of mitoses per 15,000 nuclei for these 3 mice was 13.3. The number of mitoses is slightly less than in normal epidermis, but the difference is too small to be significant.

Six hours later, at 12 midnight, 12 hours after the first application of methylcholanthrene, the ears were removed from 3 additional mice. The incidence of mitoses in the epidermis of these ears was 1, 13, and 18 mitoses per 15,000 nuclei, an average of 10.6 mitoses. There was further decrease in the number of mitoses after 12 hours.

Table I: Mitoses per 15,000 Nuclei and Percentage of Mitoses in the Epidermis of 3 Untreated Strain A Male Mice

| Mouse no. | Mitoses per 15,000 nuclei | Percentage of mitoses |
|---|---|---|
| 1 | 9 | .06 |
| 2 | 19 | .13 |
| 3 | 23 | .15 |
| Average | 17 | .11 |

Six hours later, at 6 A.M., the average number of mitoses per 15,000 nuclei for 3 mice was 25, showing some increase in the number of mitoses over the normal. Six hours later, at 12 noon, there was a slight decrease in the average number of mitoses per 15,000 nuclei for 3 mice; 22.6 as compared with 25 for 6 A.M. However, this figure is greater than that for normal epidermis.

The average number of mitoses per 15,000 nuclei for the 3 mice at 6 P.M., 12 midnight, and 6 A.M. showed a gradual increase over the previous averages. These averages were 28.6, 29.3, and 43 mitoses per 15,000 nuclei.

In the last group, the ears of 3 mice taken at 12 noon, 48 hours after the painting with methylcholanthrene, the incidence of mitoses in the epidermis was 24, 30, and 34 mitoses per 15,000 nuclei. The average number of mitoses per 15,000 nuclei was 27, which represented a decrease in the number of mitoses in the 6 hours since the taking of the previous group of ears, but which still showed an increase over the normal average. This average of 27 corresponds quite closely

to the average of 26.3 for the 3 mouse ears studied at the same interval in the previous experiment (Cooper and Reller, 2).

The individual counts for each mouse and the percentage of nuclei in mitosis are shown in Table II, and presented graphically in Fig. 1.

TABLE II: MITOSES PER 15,000 NUCLEI, PERCENTAGE OF MITOSES, AND AVERAGE NUMBER OF NUCLEI PER UNIT AREA IN THE EPIDERMIS OF STRAIN A MALE MICE DURING THE FIRST 48 HOURS AFTER A SINGLE APPLICATION OF METHYLCHOLANTHRENE

| Mouse no. | Time after painting | Mitoses per 15,000 nuclei | Percentage of mitoses | Average number of nuclei per unit area |
|---|---|---|---|---|
| 1 | 6 hours | 7 | 0.05 | 51.6 |
| 2 | 6 hours | 22 | 0.15 | 53.4 |
| 3 | 6 hours | 11 | 0.07 | 55.4 |
| Average | | 13.3 | 0.09 | 53.4 |
| 4 | 12 hours | 1 | 0.01 | 49.2 |
| 5 | 12 hours | 13 | 0.09 | 50.2 |
| 6 | 12 hours | 18 | 0.12 | 51.4 |
| Average | | 10.6 | 0.07 | 50.3 |
| 7 | 18 hours | 22 | 0.15 | 57.0 |
| 8 | 18 hours | 23 | 0.15 | 56.6 |
| 9 | 18 hours | 30 | 0.20 | 56.4 |
| Average | | 25.0 | 0.17 | 56.6 |
| 10 | 24 hours | 24 | 0.16 | 50.8 |
| 11 | 24 hours | 19 | 0.13 | 57.4 |
| 12 | 24 hours | 25 | 0.17 | 54.4 |
| Average | | 22.6 | 0.15 | 54.2 |
| 13 | 30 hours | 36 | 0.24 | 61.6 |
| 14 | 30 hours | 23 | 0.15 | 52.6 |
| 15 | 30 hours | 27 | 0.18 | 52.0 |
| Average | | 28.6 | 0.19 | 55.4 |
| 16 | 36 hours | 28 | 0.19 | 50.4 |
| 17 | 36 hours | 28 | 0.19 | 53.0 |
| 18 | 36 hours | 32 | 0.21 | 62.4 |
| Average | | 29.3 | 0.20 | 55.2 |
| 19 | 42 hours | 59 | 0.39 | 53.8 |
| 20 | 42 hours | 27 | 0.18 | 57.8 |
| 21 | 42 hours | 43 | 0.29 | 59.2 |
| Average | | 43 | 0.29 | 56.9 |
| 22 | 48 hours | 24 | 0.16 | 57.8 |
| 23 | 48 hours | 34 | 0.23 | 53.6 |
| 24 | 48 hours | 30 | 0.20 | 55.8 |
| Average | | 29.3 | 0.20 | 55.7 |

An attempt was made to measure any changes that might occur in the number of nuclei per unit area during the course of the experiment by counting the total number of nuclei included within the counter square in 5 sample areas of each ear. The square, when used in a $\times 15$ ocular with a 2 mm. oil-immersion objective, covers an area measuring 57.5$\mu$ by 57.5$\mu$. The average number of nuclei per square for the epidermis of the ears of the untreated mice was 49.8 (Table I, Cooper and Reller, 2). Six hours after the application of methylcholanthrene the number of nuclei per square increased to an average of 53.4. This increase may be accounted for by a decrease in nuclear size, or by an actual increase in the number of cells, because of epidermal hyperplasia. In Table II the average number of nuclei per unit area in the total mounts of the epidermis of the right ear of each experimental mouse is correlated with the time after painting at which the ear was removed for study. There is a slight but continuous increase in nuclei per unit area as the time after the painting increases.

### MICROSCOPIC SECTIONS OF LEFT EARS

In none of the ears was there any gross evidence of change within the first 48 hours after painting, but some microscopic variations from the normal were observed.

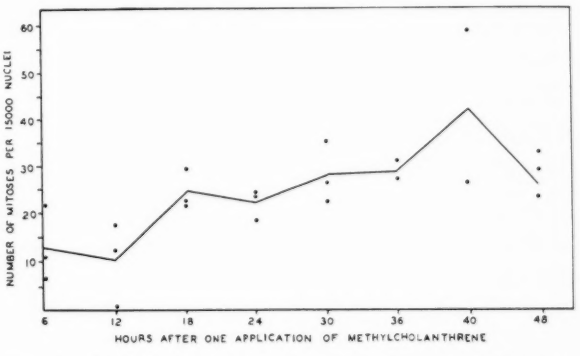


FIG. 1.—Incidence of mitoses in the epidermis of the ears of mice during the first 48 hours following a single application of methylcholanthrene.

The normal epidermis of the ear of the mouse is made up of several layers of cells. There is a narrow stratum corneum, and below this a suggestion of a granular layer that is composed of definitely flattened cells. The cytoplasm of these cells appears almost homogeneous because the granules are so densely packed. Beneath the granular layer there are from 2 to 4 rows of undifferentiated, polyhedral cells. The nuclei are large, with a moderate amount of chromatin and prominent nucleoli.

Certain changes are apparent in the epidermis within the first 48 hours after a single application of methylcholanthrene. The epithelium of the ears removed during the first 6 hours after painting was from 2 to 4 cells thick, thus not varying greatly from the normal. However, in sections of ears removed from 12 to 30 hours after painting, there were large areas where the epidermis appeared to be only 1 cell in thickness. At the same time, in other parts of the epidermis, the granular layer was more prominent. The nuclei of this layer were larger and the granules of the cytoplasm more numerous and more distinct. The stratum cor-

neum was present, but showed no deviation from the normal.

The epidermis of the ears removed from 36 to 48 hours after painting appeared thicker than either that of the treated ears removed previously or that of normal ears. The granular layer at this time was not so prominent as in the earlier stages. Here, too, the stratum corneum was present and not conspicuously altered. However, some of the hair follicles were distended by plugs of keratin, but showed no other changes. In none of the treated ears was there any evidence of ulceration.

## DISCUSSION

In the first 48 hours, the epidermis of the ears apparently reacts somewhat differently to a single application of methylcholanthrene from that of the other regions of the body. In the epidermis of the back Cramer and Stowell (4) have observed after this same time interval the differentiation of the epidermis into layers, *i.e.,* basal layer, stratum spinosum, stratum granulosum, and stratum corneum. By contrast, differentiation in the epidermis of the ears is not so complete. However, in the first 30 hours there are local decreases in thickness of the epidermis associated with an hypertrophy of the granular layer. In the next 18 hours the epidermis becomes thicker and the granular layer less conspicuous.

The difference in degree of differentiation between the epidermis of the ears and that of the back, as described by Cramer and Stowell (4), may be due to two factors: (a) Regional variations in the skin of different anatomical locations, and (b) differences due to the method of application of the carcinogen. Cramer and Stowell applied the carcinogen by a single brush stroke down the center of the back. Our application was also made by a single stroke of a similar brush down the center of the inside surface of the ear. Because of the size and structure of this organ, the whole inside surface of the ear was covered with the carcinogen at one stroke. The epidermis of the ear does not, therefore, present the differences in reaction at different distances from the brush stroke that are described by Cramer and Stowell on the back. For this reason the ear was considered more suitable for a study of mitosis, since the reaction to the carcinogen is more constant over its entire surface.

These results indicate that methylcholanthrene, when applied to the skin of the mouse ear, produces a stimulating effect on cell division in the epidermis, even within the first 48 hours after a single application. During the first 12 hours the mitotic count was a little lower than that for normal skin; but the difference was scarcely large enough to be significant. The fact that the counts made at 6 hours and 12 hours after painting were lower than normal, may be explained by differences in diurnal mitotic rhythm. In a previous study by Cooper and Franklin (1), mitotic activity in the epidermis of the mouse was found to be lowest at 10 P.M. and greatest at 10 A.M. Since these two groups of specimens were removed at 6 P.M. and at midnight, they would have been taken during the low cycle of the diurnal rhythm.

Eighteen hours after the application of methylcholanthrene, however, the incidence of mitoses rose above the normal average and remained so for the first 48 hours. At the end of 48 hours, the average number of mitoses was 27. This figure coincides quite well with the number of mitoses found at the beginning of a previous experiment (Cooper and Reller, 2), in which the incidence of mitotic figures was studied during the period of precancerous hyperplasia extending from 2 to 93 days. In this experiment the average number of mitoses encountered at 48 hours after a single painting with methylcholanthrene was 26.3. It is interesting that after the first 12 hours following painting with methylcholanthrene the diurnal rhythm in cell division found in normal mouse epidermis is interrupted. It would seem, therefore, that methylcholanthrene produces an increase in cell division in the epidermis that begins at 18 hours after application of the carcinogen. As has been shown in the previous study, this rise is progressive for the first 37 days.

## SUMMARY

1. Twenty-four mice were painted once with methylcholanthrene on the inner surfaces of the ears. Ears were removed from each of 3 mice at 6 hour intervals for 48 hours. Mitotic counts were made on total mount preparations of the epidermis of these ears. Fifteen thousand nuclei were counted in each ear, and the number of mitoses encountered was recorded.

2. At 6 and 12 hours after painting the mitotic count was slightly lower than that for normal skin.

3. Eighteen hours after painting the mitotic count rose above the normal average and remained above it for the first 48 hours.

4. Methylcholanthrene, when applied to the skin of the mouse ear, produces a stimulating effect on cell division in the epidermis within the first 48 hours after a single application.

## REFERENCES

1. Cooper, Z. K., and Franklin, H. C. Mitotic Rhythm in the Epidermis of the Mouse. Anat. Rec., **78**:1-8. 1940.
2. Cooper, Z. K., and Reller, H. C. Mitotic Frequency in Methylcholanthrene Epidermal Carcinogenesis in Mice. J. Nat. Cancer Inst., **2**:335-344. 1942.
3. Cramer, W., and Stowell, R. E. The Early Stages of Carcinogenesis by 20-Methylcholanthrene in the Skin of

the Mouse. II. Microscopic Tissue Changes. J. Nat. Cancer Inst., **2**:379-402. 1942.

4. Cramer, W., and Stowell, R. E. Skin Carcinogenesis by a Single Application of 20-Methylcholanthrene. Cancer Research, **3**:36-42. 1943.
5. Mider, G. B., and Morton, J. J. Skin Tumors Following a Single Application of Methylcholanthrene in C57 Brown Mice. Am. J. Path., **15**:299-302. 1939.
6. Page, R. C. Cytologic Changes in the Skin of Mice During Application of Carcinogenic Agents. Arch. Path., **26**: 800-813. 1938.
7. Pullinger, B. D. The First Effects on Mouse Skin of Some Polycyclic Hydrocarbons. J. Path. and Bact., **50**:463-471. 1940.
8. Simpson, W. L., and Cramer, W. Further Observations on Skin Carcinogenesis by a Single Application of 20-Methylcholanthrene. Cancer Research, **3**:604-605. 1943.
9. Wolbach, S. B. The Latent Period in Experimental Carcinogenesis. Arch. Path., **22**:279. 1936.
10. Wolbach, S. B. Responses to Carcinogenic Chemicals Antecedent to Tumor Formation. Am. J. Path., **13**:662-663. 1937.

# The Multiple Constitution of Abnormal Ciliates Produced by Blastomatogenic Agents*

J. C. Mottram, M.B.

*(From Mount Vernon Hospital, Northwood, Middlesex, and The Radium Institute, London, England)*

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It has long been known that many cancer cells are of multiple constitution. This has not been considered a fact of much importance, because multiple cells are present in normal tissues, and because it was thought that some cancer cells were of normal constitution since they contained the normal number of chromosomes. Nevertheless two recent papers go far to show that cancer cells may all be doubles or multiples. Ehrich (3), using human material, measured the volumes of the nuclei of tumor and of normal cells, using a special technic to avoid distortion by fixation, which others, in his opinion, had not controlled. Normal nuclei show a modal size distribution: If N be the size of recently divided nuclei, 2N will be the size of those about to divide. The change from N to 2N is quickly accomplished, so that nuclei of intermediate size are rare; hence the modal distribution. In some normal tissues a few nuclei of 4N size are found. In tumors, Ehrich found only nuclei of 2N or higher multiples, and none of size N. This means that all the cells of tumors are doubles or multiples.

Biesele, Poyner, and Painter (2) deal in full with all the evidence for multiple cells in tumors. They were not able completely to confirm Ehrich's findings by measuring nuclear volumes, because, in tissues pressed out under coverglasses, they could not distinguish cancer cells from connective tissue and other normal cells present in the tumors, and so recorded some cells of N size. They found that 75 per cent were 2N size and many higher multiples. By measuring mitotic volumes normal cells were avoided and here Ehrich's findings were confirmed. The volumes were 2 to 4 times the normal.

Since in many cases the number of chromosomes was normal, it follows that the chromosomes ought to be 2 or 4 times increased in size. Chromosomal volumes were therefore measured. In middle prophase, normals were 0.66 cubic microns and tumor cells 1.4, because at this stage they were twice as long. In metaphase normals were 0.5 cubic microns, tumors 1.1 cubic microns; here the increase was due to both greater length and greater width. Chromosomes 4 times increased and higher polytenes were also present in tumors.

They also discovered that these double chromosomes were produced by endomitosis. The chromosomes doubled themselves as they do during mitosis, but no spindle was formed nor did the disappearance of the nuclear membrane occur, so that the doubled chromosomes did not separate but went to form a new nucleus of twice the size, containing diplochromosomes. They maintain that all the chromosomes of the mouse cancers examined were double or higher polytenes.

Biesele (1) examined an ovarian tumor of a goldfish: the chromosomes had an average volume double those of healing skin and of normal ovary. The nucleolar number was 4 in the normals, 8 in the tumor cells. Hence the chromosomes of the tumor must be regarded as diplochromosomes.

In view of these findings, an examination of some abnormal races of ciliates produced by blastomatogenic agents has been made, because they may well be the equivalent of tumors in multicellular animals (8). These abnormals began as double organisms and many are without doubt double or multiple.

The micronucleus is much more the equivalent of nuclei in higher animals than the macronucleus, so it was decided to use it as a measure. Stained abnormals of *Colpidium sp.* were used that had been produced by exposure to ultraviolet radiation. The micronucleus is spherical, so that to obtain its volume it is only necessary to measure its diameter. This was done with a Leitz micrometer eyepiece so adjusted that readings represented tenths of a micron. The diameter of a normal micronucleus is about 5 microns, which was therefore half a turn of the micrometer screw.

Altogether 178 micronuclei were measured, present in 70 organisms and representing 23 separate abnormal races. The normal number of micronuclei is 1. In the abnormals 2 had 1 nucleus, 33 had 2 nuclei, 23 had 3, 10 had 4, and 2 had 5; 9 nuclei were dividing and so could not be measured.

* Because of the difficulties of international communication the author has not read proof of this article.

The distribution of the volumes of the nuclei is shown in Fig. 1. It is seen that the vast majority are distributed around 50 cubic microns; this is the size of the normals. There are 20 between 100 and 130 cubic microns, forming a mode representing a volume about twice the normal; some of these are probably normal micronuclei about to divide.

There is another mode of 6 nuclei at 160 to 190 cubic microns about 4 times the normal size; 4 other larger nuclei do not fit into any regular multiple of the normal. The very small nuclei of 20 cubic microns may represent aneuploids due to asymetrical mitosis. Evidently many of these abnormals are multiples not only by being multinucleated but also in having nuclei 2 to 4 or more times the normal size.

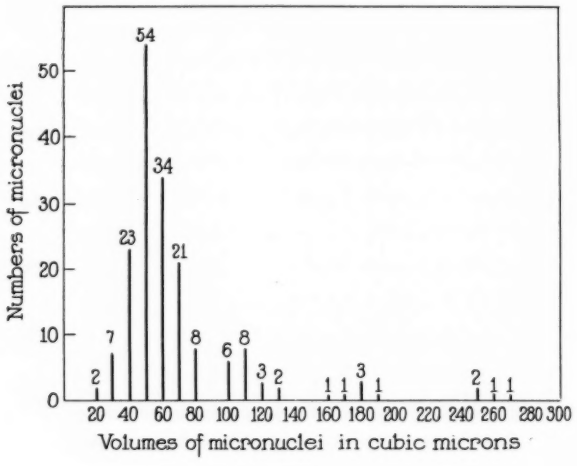


FIG. 1

Some however could be normal organisms, namely 2 having 1 micronucleus and 33 having 2.

Those with 2 nuclei could be normals, because after the micronucleus has divided a short time elapses before the macronucleus and the cytoplasm begin to pinch off into two. In this case the micronuclei lie at the two ends of the cell, away from the macronucleus. If, on the other hand, the organism is a double then the 2 micronuclei lie close to the macronucleus and close together near the centre of the cell.

Of the 33 organisms with 2 micronuclei, 30 were doubles and 3 were normals undergoing division.

The nuclei of the 2 containing a single micronucleus measured 102 and 258 cubic microns; the first could be a normal about to divide; the other must be a multiple individual.

It follows that out of 70 organisms examined, 4 were probably of normal constitution as regards their micronuclei.

Outline drawings of normal and abnormal clones of *Colpidium sp.* are available for estimation of their volume and so of their constitution, whether single or multiple. The volumes were calculated in the usual way from measurements of major and minor axes. Owing to the irregular shape of some of the abnormals, their volumes are less accurate than the normals.

In the experiments 2 cultures were grown from 2 sister organisms; 1 was treated with a blastomatogenic agent, the other served as a control. When an abnormal was found in the treated culture it was picked out and grown in normal media to about 100 individuals. A control was also picked out and treated alike. The organisms were then killed with "Susa" fixative and 50 of each chosen at random were drawn to scale. The volume distribution of abnormals produced by exposure to radiation, and of controls, are shown in Fig 2. The normals range about 6,000 cubic microns, the abnormals about 12,000 cubic microns with some of much larger size. The wide

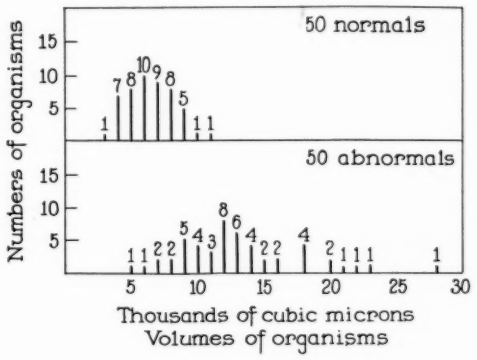


FIG. 2

spread of the abnormals is probably due, as already mentioned, to their irregular shape, which made calculations of volume less accurate than for the normals.

In a second experiment (Fig. 3), 3 abnormals produced by cold are compared with one control. A different species of *Colpidium* was used. In A and B there is a decided shift to the larger size. In A no regular multiples are to be made out. In B there are uncertain peaks at 6, 9, and 12 thousand microns, representing 2, 3, and 4 times the normal volume of 3000 cubic microns.

In C it is evident that organisms of normal volume are present, there being a decided peak at 3000 cubic microns. Some very large individuals occur but no regular increases are shown.

These findings and those with micronuclei support the contention of protozoologists that among the abnormals some normals are often to be found and that by selection clones of normals can be outbred; likewise, of course, by selection, clones composed entirely of abnormals. It is not possible to say with

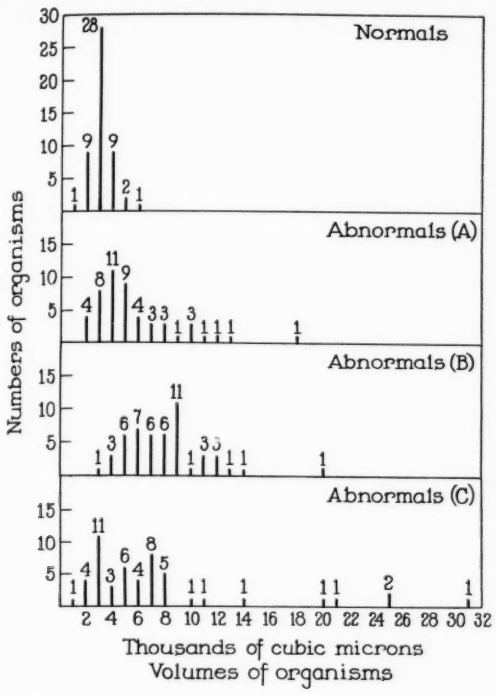


FIG. 3

DISCUSSION

If the cells of early primary tumors could be cultured from single cells, it might be found that some cells were normals and that others varied in departure from the normal up to nonviable monsters. There is evidence of variability in tumor cells. When primary skin tumors of mice are autografted as minute pieces one obtains from the same tumor simple epithelial cysts and epitheliomas; or epitheliomas of different growth rate, or of different differentiation, or of varying malignancy indicating that the tumors were composed of cells not all alike (4—6). The study of the growth curves of these primary tumors supports this (7), and of cancer cells free in pleural effusions (9). Thus are found in primary tumors populations of cells closely resembling the clones of abnormal ciliates.

In secondary tumors, and especially in inoculated tumors, such variation on inoculation is not found; microscopically the cells show little variation, presumably because the fastest growing ones have had time to outgrow those of slower growth rate, so that one class of cell comes to form the whole of the tumor. The environment is the selecting agent, corresponding to the protozoologist, who by selection produces races of ciliates differing in degree of abnormality.

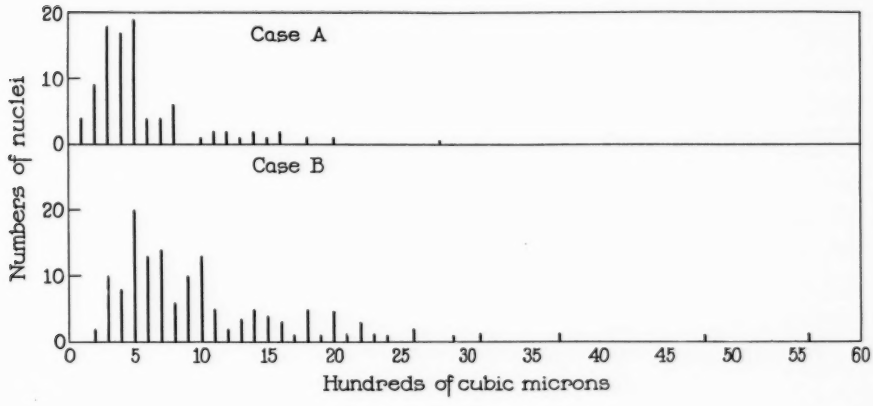


FIG. 4

this material whether the large micronuclei represent polyploidy or polytene chromosomes; the chromosomes are too small and dust-like.

In order to compare these cells with the cells of tumors, measurements of the volumes of the nuclei of 2 cases of carcinoma of the human lung were made (Fig. 4). As the cells were free in pleural exudates, films could be made. In calculating the volumes of the nuclei it was assumed that the nuclei collapsed without distortion on their minor axis; this assumption is doubtful, but at any rate the range of their volumes is well shown and their resemblance to abnormal ciliates is seen by comparison with Figs. 2 and 3.

In Case B the smallest nuclei are very large, so that the cells could be all of double or multiple constitution. In Case A the smallest nuclei are within normal range.

There is a want of unanimity over tumor cells as regards their size, the volume of their nuclei, the number of their nucleoli, their chromosomes, their centrosomes, etc. For instance, Biesele and others (2) found that all the nuclei of a number of inoculated mouse tumors contained only diplochromosomes or higher polytenes; Ehrich (3), that the nuclei of human tumors were all of double or higher volume. Others do not find these uniformities. Perhaps these differences can be explained by some having worked with primary tumors and others with inoculated tumors.

Mention may here be made of another fact of tumor genesis, which appears to support the contention that abnormal races of ciliates are the equivalent

of tumors in higher animals. Sub-effective exposures to blastomatogenic agents can be made effective by subsequent treatment with agents that cause hyperplasia but not neoplasia.

Presumably an altered cell, or cells, can be developed into a tumor by being made to undergo reproduction. In ciliates the altered cell consists of a double individual; but a simple double cell cannot be the first tumor cell, since double cells often occur in normal tissues. If, however, the double ciliate be picked out and made to reproduce rapidly, it often happens that an abnormal race results. A double cell is prone to divide irregularly so that trebles, quadruples, and higher multiples are formed, as well as some normal singles; further, irregular multiples also occur.

By this means opportunity is given for the production of cells departing from the normal to such a degree as to have the constitution required for tumor cells. The consideration of the production of abnormal ciliates would thus give a ready explanation for the remarkable fact that tumors can be developed by causing hyperplasia of cells previously exposed to blastomatogenic agents.

The fact that tumors can be produced by a great variety of agents favors the view that the specific change is not brought about directly, as for instance by gene mutation, but by a secondary change that requires to be developed.

## CONCLUSION

(1) Measurements of individuals and of micronuclei of normal *Colpidium sp.* and of abnormals produced by blastomatogenic agents showed that the majority were of varying multiple constitution, and a few were singles.

(2) This confirmed previous findings in which, by selection from abnormal races, clones of apparently normal individuals were produced.

(3) The cells of primary tumors are considered from the point of view that the abnormal races of ciliates are equivalent to tumors in multicellular animals; support for this contention is found.

## REFERENCES

1. Biesele, J. J. Diplochromosomes in a Goldfish Tumor. Cancer Research, **3**:411-412. 1943.
2. Biesele, J. J., Poyner, H., and Painter, T. S. Nuclear Phenomena in Mouse Cancer. University of Texas Publication No. 4243. Austin: University of Texas, 1942.
3. Ehrich, W. E. Nuclear Sizes in Growth Disturbances, with Special Reference to Tumor Cell Nucleus. Am. J. M. Sc., **192**:772-790. 1936.
4. Mottram, J. C. On the Correlation Between Malignancy and the Rate of Growth of Tar Warts in Mice. Am. J. Cancer, **22**:801-830. 1934.
5. Mottram, J. C. On the Origin of Tar Tumours in Mice, Whether from Single or Many Cells. J. Path. & Bact., **40**:407-414. 1935.
6. Mottram, J. C. The Relation Between the Growth Rate of Tar Warts in Mice and Their Corresponding Autografts. J. Path. & Bact., **42**:79-90. 1936.
7. Mottram, J. C. A Further Consideration of the Growth Rates of Tar Warts in Mice and Their Autografts. Am. J. Cancer, **28**:115-120. 1936.
8. Mottram, J. C. The Problem of Tumours. The Application of Blastogenic Agents to Ciliates, a Cytoplasmic Hypothesis. London: H. K. Lewis and Company, Ltd., 1942.
9. Mottram, J. C. Observations on the Morphology of Tumour Cells. J. Path. & Bact., **54**:511-514. 1942.

# The Ascorbic Acid Content of the Liver in Mice*

E. L. Kennaway, N. M. Kennaway, and F. L. Warren**

[*From The Chester Beatty Research Institute, the Royal Cancer Hospital (Free), London*]

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This investigation was suggested by the data published by Fox and his associates (7, 8) upon the comparative ascorbic acid content of the livers of Europeans, and of Bantu natives, living in South Africa. The livers of the Bantu contained a lower concentration of ascorbic acid; as yet we do not know whether this difference is due to racial or to environmental factors, nor whether it is related to the far higher incidence of primary cancer of the liver in the Bantu. The ascorbic acid content of the liver is of interest also in connection with the destruction of estrogens in this organ described by B. Zondek and later workers (2; bibliography), and with the action of ascorbic acid on polycyclic hydrocarbons (12).

The mice, numbering 282, belonged to the following classes: (a) dba, C3H, RIII (high mammary cancer, pure lines); (b) CBA, C57 (low mammary cancer, pure lines); (c) one of three strains, brown, buff, and white MRC, maintained by the Medical Research Council, which are inbred but not pure lines, in which there has been no brother-to-sister mating (4); (d) mice of mixed stock obtained from a dealer.

The mouse was killed by dislocation of the neck; the liver was weighed and ground with sand before and after the addition of 10 ml. of 3 per cent metaphosphoric acid; the mixture was centrifuged, and the solid residue mixed and centrifuged three more times with fresh portions (8, 5, and 5 ml.) of acid, or 28 ml. in all. Many experiments showed that further extraction did not produce significant amounts of titratable material. The extract was titrated with 2,6-dichlorophenolindophenol (about 0.010 per cent) standardized by means of ascorbic acid and iodine; a mouse liver requires usually between 5 and 10 cc. of the solution.

All the pure-line mice were bred here, and had therefore been in the same environment throughout life; various temporary changes were made in the diet (generally made up of oats, bread, Millard Marsh diet, dog biscuits, a proprietary food "Cake Nuts,"[1] cod liver oil, and marmite) in part owing to the restrictions of wartime, but none of the variations in ascorbic acid content could be traced to these changes, so they are not recounted here.

## RESULTS

The mean results, classified according to strain, sex, and age, are summarized in Table I. The age factor is represented in the table by separation at the 200th day of life into younger and older groups: the effect of age is shown in detail by graphs of the individual points (Figs. 1 to 4). The choice of the 200th day is of course quite arbitrary, but this time is probably near the middle of the most active period of reproduction; according to Snell (10) the first fertile mating commonly occurs at 7 to 10 weeks, while "the most useful breeding period of most inbred females terminates when they reach ten or twelve months of age, for though litters may continue to be produced after this, breeding is apt to be irregular and the litters small. . . . . Males will usually breed several months longer than females of the same stock."

*Strain.*—1. The mean concentrations of ascorbic acid in the liver are greater in the three high-cancer strains dba, RIII, and C3H (range of means 322 to 467 $\gamma$ per gm. liver) and in the males of the low-cancer strain CBA (means 344 to 356 $\gamma$) than in the females of the CBA strain (means 219 to 307 $\gamma$) and in both sexes of the other low-cancer pure-line C57 (means 175 to 216 $\gamma$).

In order to reduce the number of graphs, all the data from the three high-cancer strains have been pooled in Fig. 1. By far the highest amounts were found in the dba females; unfortunately figures for four mice only are available as our stock has shown reduction in breeding capacity in addition to other changes (5).

The data given above for 20 male CBA mice are to some extent amplified by estimations on a further series of 37 that received subcutaneous and intraperitoneal injections of sesame oil as controls in ex-

* Because of the difficulties of international communication the authors have not read proof of this article.

** One of us (F.L.W.) wishes to express his thanks to the Sir Halley Stewart Trust for a Fellowship held during the course of this work. We are indebted to Mr. P. J. Ewers for his records of the life histories of the mice under his care. This investigation has been assisted by generous financial grants from the British Empire Cancer Campaign and The Anna Fuller Fund.

[1] Supplied by the North-Eastern Agricultural Co-Operative Society, Ltd., Aberdeen, Scotland.

periments on carcinogenic compounds that will be published shortly. We had expected that this solvent would be indifferent, but the results (Table I) show that the oil lowers the concentration of ascorbic acid from a mean of 349 γ to one of 307 γ, *i.e.*, by 12 per cent. This fall in concentration of ascorbic acid appears to be due, not to a diminution in the absolute amount, but to enlargement of the liver. The liver of the average normal CBA male makes up 4.8% of the body weight (Table I); if the percentage were increased, as in the injected mice, to 5.4 simply by the addition of water or fat, for example, the concentration of ascorbic acid would be lowered from the normal level of 349 γ per gm. to 312 γ, which is very close to the actual mean amount found, namely, 306 γ. We have under investigation other data on this enlargement of the liver after the injection of sesame, and of arachis, oil.

TABLE I

| Strain | Sex | Number | Age, days | Mean weight, gm. Body | Mean weight, gm. Liver | Liver weight per cent of body weight | Mean ascorbic acid in liver: γ per gm. liver (range in brackets) | Mean ascorbic acid in liver: γ per gm. body weight | Mean ascorbic acid in liver: Total | Standard deviation | Standard error of mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| dba | ♂ | 8 | 80–200 | 19.30 | 1.14 | 5.95 | 389 (253–520) | 23.1 | 437 | 83.6 | 29.6 |
| | | 20 | 201–544 | 24.00 | 1.30 | 5.40 | 325 (217–393) | 17.5 | 412 | 43.0 | 9.6 |
| | | all | | 22.60 | 1.26 | 5.60 | 344 | 19.1 | 419 | | |
| | ♀ | 4 | 314–425 | 28.70 | 1.95 | 6.70 | 467 (407–532) | 31.2 | 893 | 51.2 | 25.6 |
| C3H | ♂ | 13 | 117–200 | 21.70 | 1.06 | 4.90 | 409 (357–480) | 20.2 | 433 | 45.3 | 12.6 |
| | | 30 | 201–544 | 24.20 | 1.23 | 5.10 | 378 (226–467) | 19.2 | 469 | 56.1 | 10.2 |
| | | all | | 23.40 | 1.18 | 5.00 | 387 | 19.5 | 458 | | |
| | ♀ | 30 | 248–550 | 23.30 | 1.37 | 5.90 | 322 (232–483) | 18.9 | 447 | 67.1 | 12.0 |
| RIII | ♂ | 12 | 49–200 | 18.10 | 0.92 | 5.16 | 342 (278–403) | 17.6 | 317 | 51.4 | 14.8 |
| | | 5 | 201–320 | 21.60 | 1.12 | 5.15 | 368 (278–446) | 18.8 | 404 | 61.9 | 27.7 |
| | | all | | 19.10 | 0.98 | 5.16 | 349 | 17.9 | 343 | | |
| | ♀ | 12 | 36–200 | 15.20 | 0.84 | 5.50 | 370 (254–499) | 19.9 | 299 | 73.1 | 21.1 |
| | | 3 | 256–320 | 23.40 | 1.54 | 6.50 | 323 (275–367) | 21.2 | 495 | 46.1 | 26.6 |
| | | all | | 16.80 | 0.98 | 5.70 | 360 | 20.1 | 338 | | |
| CBA Uninjected | ♂ | 11 | 110–169 | 27.20 | 1.33 | 4.90 | 344 (236–432) | 16.8 | 461 | 58.7 | 17.7 |
| | | 9 | 233–375 | 26.90 | 1.25 | 4.65 | 356 (310–436) | 16.6 | 447 | 36.2 | 12.1 |
| | | all | | 27.05 | 1.30 | 4.80 | 349 | 16.7 | 455 | | |
| Injected (Sesame oil subcutaneous and intraperitoneal) | | 19 | 138–200 | 23.50 | 1.28 | 5.47 | 310 (226–478) | 16.8 | 394 | 54.4 | 12.5 |
| | | 18 | 210–268 | 25.10 | 1.34 | 5.32 | 301 (221–379) | 16.0 | 399 | 46.2 | 10.9 |
| | | all | | 24.30 | 1.31 | 5.40 | 306 | 16.4 | 397 | | |
| | ♀ | 16 | 52–200 | 18.60 | 1.05 | 5.70 | 307 (246–429) | 17.6 | 316 | 45.5 | 11.4 |
| | | 21 | 201–581 | 24.20 | 1.42 | 5.85 | 219 (149–363) | 12.8 | 314 | 60.9 | 13.0 |
| | | all | | 21.80 | 1.25 | 5.80 | 257 | 14.8 | 315 | | |
| C57 | ♂ | 8 | 99–548 | 23.20 | 1.41 | 6.10 | 216 (149–313) | 13.1 | 304 | 53.7 | 19.0 |
| | ♀ | 7 | 143–568 | 20.90 | 1.35 | 6.40 | 175 (119–278) | 11.4 | 247 | 56.3 | 21.3 |
| Buff MRC | ♀ | 9 | 120–200 | 24.00 | 1.53 | 6.40 | 191 (118–270) | 12.0 | 288 | 46.2 | 15.4 |
| | | 12 | 201–402 | 29.40 | 1.80 | 6.13 | 185 (143–270) | 11.2 | 330 | 37.4 | 10.8 |
| | | all | | 27.10 | 1.68 | 6.20 | 188 | 11.6 | 312 | | |
| Stock | ♂ | 8 | adult | 31.70 | 1.55 | 4.90 | 221 (162–279) | 10.8 | 344 | 35.6 | 12.6 |
| | ♀ | 7 | 107–200 | 26.10 | 1.46 | 5.70 | 224 (178–282) | 13.0 | 326 | 43.3 | 16.4 |

2. The two classes of mice that are not purebred (buff MRC and stock) show mean concentrations of ascorbic acid in the liver that are still lower (range of means, 185 to 224 γ) than those found in the low-cancer pure lines. All the 12 MRC mice over 200 days old had received intraperitoneal injections of saline or arachis oil, but the mean value for this group (185 γ) is practically the same as that for the younger mice (191 γ). The following are the available data about the occurrence of mammary cancer in these mice: (a) Argyll Campbell (6) says that in the female MRC mice, under conditions where "breeding was almost negligible," the percentage incidence was 6.9 (brown), 2.9 (white), and 3.7 (buff). (b) The breeder of the stock in question writes that mammary cancer occurs in mice that have had 5 to 8 litters and are 18 to 24 months old, but is otherwise very rare; among such mice the incidence is about 10 per cent. The age mentioned is more advanced than that at which a large proportion of the mammary tumors occur in pure high-cancer lines.

The range of these amounts in any one class, shown in brackets in Table I, is round about 100 per cent. Two quite exceptionally low values (65 γ in a female C57, and 78 γ in a female MRC) were excluded from the averages (Fig. 4).

*Age.*—If $P < 0.01$ is taken as the standard of significance, a difference between mice of the same sex and strain but of different ages occurs only in CBA females, in which there is a significant drop with advancing age (from a mean of 307 γ in the first 200 days to 219 γ later on; Fig. 3).

*Sex.*—Sexual differences occur in three groups. In mice over 200 days of age the males have a significantly

higher ascorbic acid content of the liver in two strains, namely, CBA (Figs. 2 and 3) and C3H, and a lower content in the dba strain. No male buff MRC mice were examined. No constant differences were found between mice that had had no, or from one to five, litters. All data from pregnant mice have been excluded from the tables, as very high values are found

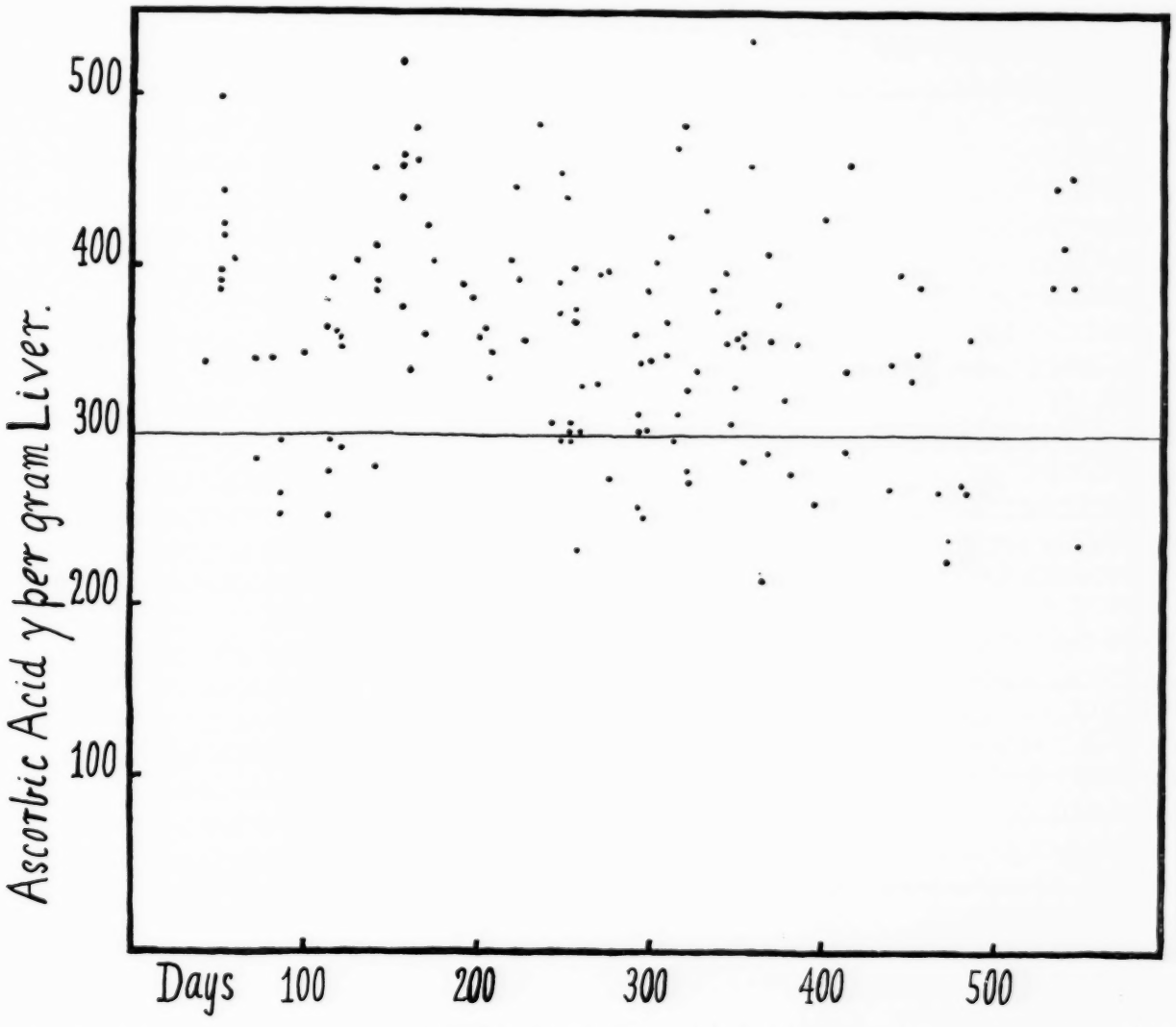


FIG. 1.—High-cancer strain mice (dba, C3H, RIII) of both sexes. To facilitate comparison of Figs. 1 to 4 a horizontal line is drawn at the 300 γ level.

sometimes in this condition; the subject is still under investigation.

## DISCUSSION

No assertion is made here that these results throw any direct light upon the incidence of either mammary or hepatic cancer in different strains of mice; the question is a complex one and requires much further work. Certainly the three strains especially liable to mammary cancer, and the males of the CBA low-cancer strain, show a higher concentration of ascorbic acid in the liver than do the other groups. But other modes of approach to this problem may of course fail to show any such differences. Thus Twombly and Taylor (11) examined the destruction of estradiol by the livers of mice of four strains (dba, A, D, C57) and observed no differences that could be correlated with the incidence of mammary cancer. The authors conclude that "The varying incidence of mammary cancer in mice cannot be explained on the basis of differences in this function of the liver." However, one might question whether the complete finality of this conclusion can be justified by a single type of experiment (incubation of slices of mouse liver weighing 0.125 to 0.15 gm. with 3 γ estradiol for one hour, and subsequent injection into rats) to which there are certainly some alternatives. A negative experiment *in vitro* last-

ing one hour may not dispose finally of a question concerning changes that, in the living mouse, may extend over many months.

The tendency for the ascorbic acid to fall with advancing age might of course suggest a lessened destruction of carcinogenic factors in the liver, even though this fall may be greater in some low-cancer than in the high-cancer strains. The complexity of the factors, the condition seen in C3H mice, and that found in the Bantu (high incidence of tumors, and low ascorbic acid, in the liver); in the latter instance our ignorance of the part played by extrinsic or intrinsic factors, or by both, makes the question still more difficult.

The female CBA mice show a significant fall with advancing age (from mean 307 $\gamma$ to 219 $\gamma$). In the only females of a high-cancer strain with which a comparison is possible (RIII, fall from mean 370 $\gamma$ to 323 $\gamma$) the decrease is not statistically significant. Warren (unpublished observations) finds another contrast between the females of these two strains; an increase of the non-heme iron of the kidney occurs in older mice, and this increase is much greater in the females of the CBA than in those of the RIII strain. Investigations upon a possible connection between these two sets of changes are in progress.

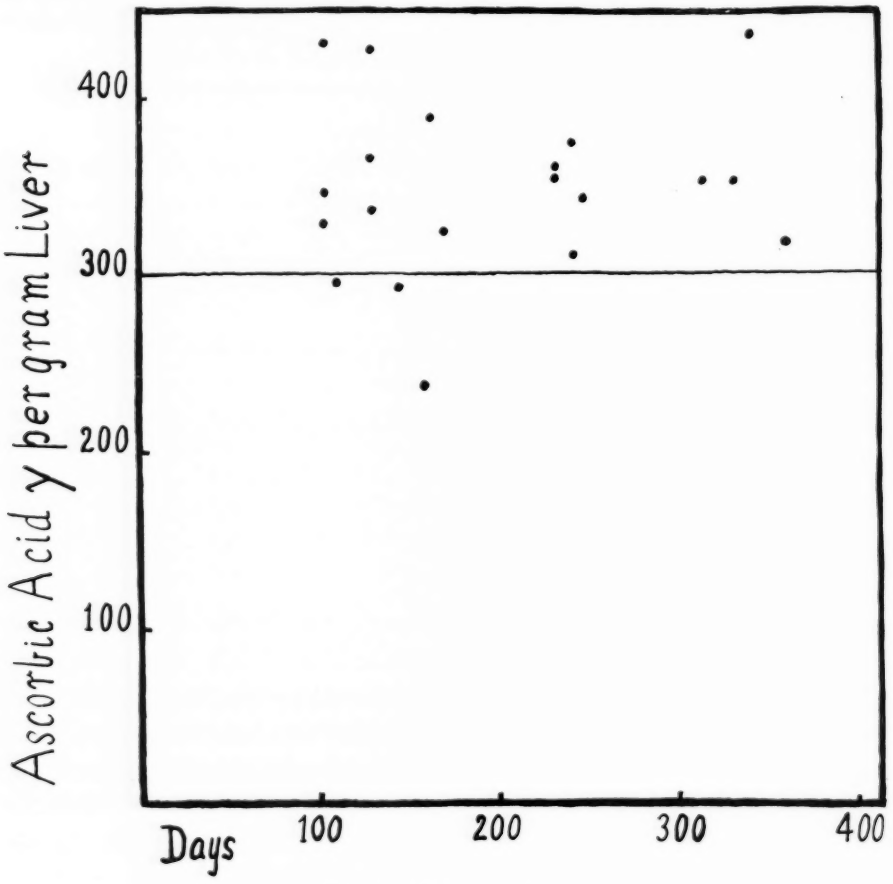


FIG. 2.—CBA male mice.

at least three in number, concerned in mammary cancer make it unlikely that any simple picture of the whole mechanism can be obtained as yet. Further observations are required on the high-cancer strains.

C3H mice are especially liable to spontaneous hepatoma, which occurs in 27 per cent of males and 10 per cent of females in the second year of life (1); we have no exact observations upon our own mice of this strain, but doubt very much whether the incidence is so high here. However, C3H mice are resistant to the induction of hepatic tumors by *o*-aminoazotoluene. These mice show a high concentration of ascorbic acid in the liver, but this is of the same order as that seen in dba and RIII mice, in which no especial frequency of tumors of the liver has been recorded. Again, we do not know what is the relation between

## GLUTATHIONE

In these analyses the titration of ascorbic acid with dichlorophenolindophenol was followed by titration of the remaining reducing substance, which is usually regarded as glutathione, with 0.01 *N* iodine (9). In

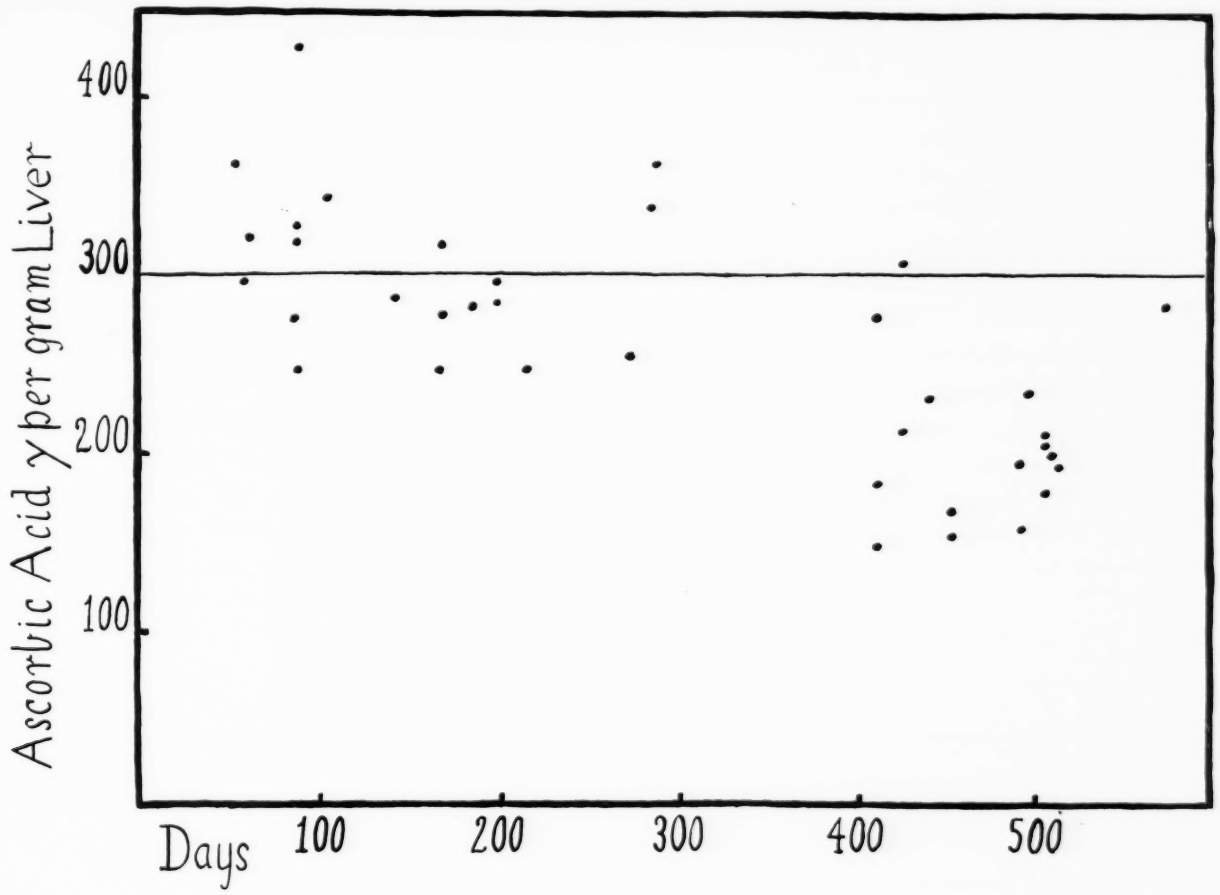


FIG. 3.—CBA female mice.

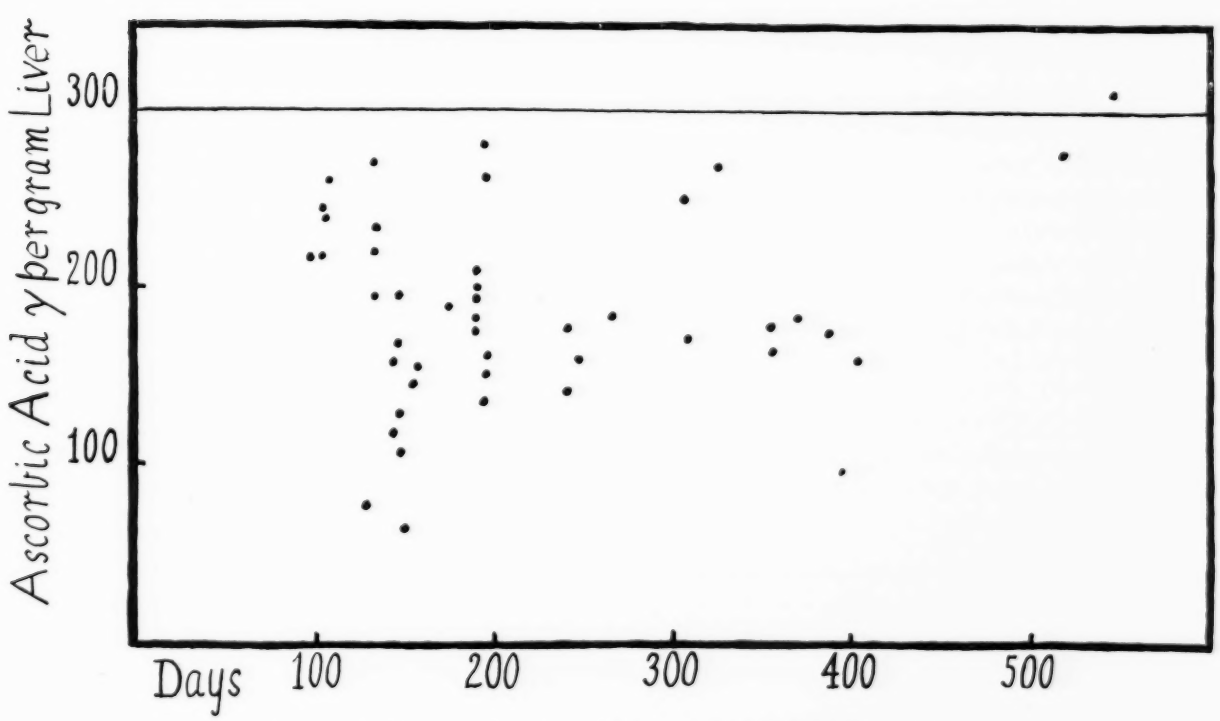


FIG. 4.—C57 and stock mice of both sexes, and buff MRC females.

TABLE II: GLUTATHIONE IN THE LIVER

| Male | | | Female | | |
|---|---|---|---|---|---|
| Strain | Age, days | Glutathione as cc. 0.01 *N* iodine per gm. liver | Strain | Age, days | Glutathione as cc. 0.01 *N* iodine per gm. liver |
| dba | —200 | 1.06 | dba | 314— | 0.82 |
| " | 201— | 1.02 | C3H | 248— | 0.84 |
| C3H | 117— | 0.97 | RIII | —200 | 1.03 |
| " | 201— | 0.87 | " | 256— | 0.80 |
| RIII | —200 | 1.15 | CBA | —200 | 0.90 |
| " | 201— | 1.00 | " | 201— | 0.82 |
| CBA | —200 | 0.99 | C57 | — | 1.08 |
| " | 201— | 1.01 | Buff MRC | —200 | 0.90 |
| C57 | — | 0.89 | " " | 201— | 0.81 |
| Stock | — | 0.76 | Stock | — | 0.77 |
| Mean | | 0.97 | Mean | | 0.88 |

TABLE III: AVERAGE LIVER WEIGHT PER CENT OF BODY WEIGHT

| | dba | RIII | C3H | CBA | C57 | MRC | | | Stock |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Mixed | Buff | White | |
| Male | 5.6 | 5.16 | 5.03 | 4.80 | 6.1 | 5.4 | — | — | 4.9 |
| Female | 6.7 | 5.70 | 5.90 | 5.76 | 6.4 | — | 6.2 | 6.3 | 5.7 |

Table II the mean amounts of glutathione for the groups of mice as classified in Table I are shown. The amounts per gm. liver, expressed in cc. 0.01 *N* iodine, range between 0.80 to 1.08 in all groups except the stock mice, in which the figure is somewhat lower (0.765 cc.). This range is in close agreement with that found by Boyland (3) and Boyland and Mawson (4) in the mice of mixed stock used by them, namely, 0.88 to 1.14 cc.

The mean concentrations of glutathione in the liver show no contrast between the high-cancer and low-cancer strains such as is found in the case of ascorbic acid, and they hence provide an additional suggestion of the special importance of the latter compound from the present point of view.

## RATIO OF LIVER WEIGHT TO BODY WEIGHT

In the course of these and some other estimations a number of data upon the ratio of liver weight to body weight have been obtained. This ratio is in all cases higher in the female mice (Table III).

## SUMMARY

The mean concentration of ascorbic acid in the liver was found to be greater in mice of three high-cancer, pure lines (dba, C3H, RIII), and in the males of the low-cancer pure line CBA, than in females of the CBA line and in both sexes of the low-cancer, pure line C57 and of two other low-cancer breeds (buff MRC and stock).

## REFERENCES

1. ANDERVONT, H. B., GRADY, H. G., and EDWARDS, J. E. Induction of Hepatic Lesions, Hepatomas, Pulmonary Tumors, and Hemangio-Endotheliomas in Mice with *o*-Aminoazotoluene. J. Nat. Cancer Inst., **3**:131-153. 1942.
2. BISKIND, M. S., and BISKIND, G. R. The Effect of Vitamin B Complex Deficiency on Inactivation of Estrone in the Liver. Endocrinology, **31**:109-114. 1942.
3. BOYLAND, E. A Note on Glutathione and Vitamin C in Tumour Tissue. Biochem. J., **27**:802-805. 1933.
4. BOYLAND, E., and MAWSON, E. H. Changes in the Livers of Mice after Administration of 3:4:5:6-Dibenzcarbazole. Biochem. J., **32**:1460-1466. 1938.
5. BURROWS, H. Decline in the Incidence of Mammary Cancer in Mice of an Inbred Strain. Cancer Research, **1**:121-122. 1941.
6. CAMPBELL, J. ARGYLL. The Incidence of Primary Lung Tumours in a Mixed Strain of Mice. Brit. J. Exper. Path., **23**:191-201. 1942.
7. FOX, F. W., and DANGERFIELD, L. F. Scurvy and the Requirements of Native Mine Labourers for the Antiscorbutic Vitamin—An Experimental Study. Proc. Transvaal Mine Medical Officers' Assoc., **19**:267-288. 1940.
8. FOX, F. W., DANGERFIELD, L. F., GOTTLICH, S. F., and JOKL, E. Vitamin C Requirements of Native Mine Labourers: An Experimental Study. Brit. Med. J., **2**: 143-147. 1940.
9. MAWSON, C. A. The Influence of Animal Tissues on the Oxidation of Ascorbic Acid. Biochem. J., **29**:569-579. 1935.
10. SNELL, G. D. Biology of the Laboratory Mouse. By the Staff of Roscoe B. Jackson Memorial Laboratory. Philadelphia: The Blakiston Company. 1941, p. 59.
11. TWOMBLY, G. H., and TAYLOR, H. C. Inactivation and Conversion of Estrogens in Vitro by Liver and Other Tissues from Human Cancer Patients and from Mice of Strains Susceptible to Mammary Carcinoma. Cancer Research, **2**:811-817. 1942.
12. WARREN, F. L. Aerobic Oxidation of Aromatic Hydrocarbons in the Presence of Ascorbic Acid. Biochem. J., **37**:338-341. 1943.

# Effects of a Low Lysine Diet on the Growth of Spontaneous Mammary Tumors in Mice and on the $N_2$ Balance in Man*

R. A. Kocher, Lt. Col., MC, A.U.S.

*(From the Laboratory of The Grace Deere Velie Foundation, Carmel, California)*

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The dietary requirement for certain amino acids essential to normal nutrition has long been established. It remained for Osborne and Mendel to distinguish those essential for normal growth from those required for maintenance. Of the 22 known amino acids that in various proportions go to make up the complex structure of the protein molecule, some 9 or 10 must be supplied to the organism in the diet. The animal is not dependent for the remainder preformed in the diet. Osborne and Mendel clearly demonstrated that while lysine is a normal constituent of body proteins its requirement for maintenance in the ordinary wear and tear of adult tissues is minimal. However, the conditions for rapid synthesis of new tissue protein incident to normal growth greatly increase the requirement for this particular amino acid. In short, they found that if lysine in the diet of healthy growing rats was reduced to the lowest level required for maintenance of fully mature rats, growth ceased; when lysine was added to such diets, growth was resumed. Thus lysine was distinguished as essentially a growth factor.

With these facts established for normal growth the question arose whether malignant tumors, which are characterized by a rapid synthesis of new protein, follow this same pattern. If the growth of neoplastic tissues can be retarded by restricting or eliminating the intake of these growth factors in the diets of the host a new weapon would be available for controlling these abnormal growths.

This problem was first outlined by this writer in 1915 (2). As a preliminary to our investigation of this problem, a quantitative analysis was made of various types of malignant tumor tissue for their content in lysine and related amino acids. It was found that the yields of lysine, arginine, and histidine were considerably higher than those obtained on hydrolysis of normal somatic tissue. At the time this work was carried out in 1915 no laboratory animals suitable for experimental work on this problem were available. In lieu of such desirable preliminary experiments on animals we carried out limited experiments on human cancer patients. Gliadin, one of the proteins of wheat, with a very low lysine content was used as the sole source of protein. Calory and vitamin requirements were supplied by starch, butter, lettuce, and orange and tomato juice. In spite of the seemingly favorable results in the few cases studied this work had to be discontinued because of lack of facilities for preparing gliadin in sufficient quantities, and was not reported in detail. Of record is the following: "An effort has been made to feed certain human cancer cases on a diet free from these substances (hexone bases) with the hope that the cancer tissue might be starved out. Its needs for growth are probably proportional to the rapidity of its extension throughout the body" (5).

More recently, with the development of pure strains of animals, particularly those showing spontaneous malignant tumors, interest in this phase of the nutrition of malignant tumors has been revived by the work of Courrier and Coste in France (1), and of Voegtlin and Thompson (8) and Voegtlin and Maver (7) in this country. Courrier and Coste fed rats bearing the Jensen sarcoma on a diet in which gliadin formed the chief source of protein. They reported a definite retardation of tumor growth in rats fed on this diet and attributed it to the low lysine content of the diet. Voegtlin and Maver have criticized this work on the ground that the authors failed to prove a growth response by the addition of lysine to the diet following the period of retardation. Voegtlin and Maver, using the Marsh strain of mice in which over 90 per cent of the females develop spontaneous mammary carcinoma, added the refinement to their technic of supplying pure crystalline lysine to the diets following the period of retarded growth on the gliadin diet and observed renewed growth, thus proving that lysine was the limiting factor. In subsequent experiments Voegtlin and Johnson (6), using the same strain of mice, found that the amino acids cystine and methionine, as well as glutathione, when reduced to a low level in the diet, caused a retardation in tumor growth; when these

* Because of the difficulties of international communication the author has not read proof of this article.

were again added to the diet growth continued at the previous rate.

From this work Voegtlin and his co-workers concluded that normal growth of young mice and the growth of spontaneous mammary carcinoma in adult mice is inhibited by a diet deficient in either lysine or cystine.

## EXPERIMENTAL

If, as appears to be true from the experiments of Voegtlin and his associates, malignant tumor growth in mice can be retarded by diets lacking in certain amino acids, the obvious next step would be to find out by similar experiments whether malignant growth can be arrested or retarded in human beings. However, the diets used thus far with mice are not suitable for human consumption. It should be shown by actual experiment, first, that a diet suitable for human consumption and lacking the lysine necessary for growth is capable of maintaining nutritive equilibrium in normal subjects; second, that such a diet fed to tumor mice is able to arrest or retard the growth of malignant neoplasms. If these two conditions were satisfactorily supported by experimental evidence a third step would be worth while; namely, a clinical trial of the diet on human subjects suffering from malignant growths.

In the present experiments the first two of these procedures were carried out. First, a diet suitable for human consumption was devised that would be palatable, adequate in calories, minerals, and vitamins, sufficient for maintenance of nitrogen balance, but lacking in one of the amino acids essential for growth; namely, lysine. This diet was tried experimentally on two healthy human subjects to determine whether nutritive requirements were met, by quantitative measurement of nitrogen balance.

Secondly, when it had been determined that nitrogen equilibrium in healthy persons could be maintained on such a diet, a similar regimen was fed to mice to determine its maintenance value, its effect on normal growth, and its effect in arresting or retarding the growth of malignant tumors. The following experiments fall into two parts with the above plan in view: Part I, experiments on healthy human subjects, and Part II, experiments with normal and with tumor-bearing mice.

## PART I

### Experiments with Low Lysine Diet on $N_2$ Balance in Normal Human Subjects

In devising a diet that is adequate in all respects except for the amount of lysine contained, the most important constituent is the proteins. Those used must have in the aggregate enough of the necessary amino acids for maintenance and must at the same time be deficient in lysine. Two naturally occurring vegetable proteins were considered; namely, gliadin and zein. Gliadin from wheat is somewhat tedious to prepare in quantity, whereas zein has been available in the market under the trade name of maizite, supplied by the American Maizite Products Company, Roby, Indiana.

This product is entirely soluble in 80 per cent alcohol. Because of the possibility that traces of globulin and glutelin might be present, the zein used in these experiments was first extracted with dilute sodium chloride solution to remove possible traces of globulin, then dissolved in 80 per cent alcohol and reprecipitated from this solution by the addition of water. Traces of oily substances present that made the zein somewhat unpalatable were satisfactorily removed during the process of baking zein crisps (cookies) in very thin layers in the oven at a low temperature for one hour.

As zein is entirely lacking in lysine and is very low in tryptophan content, it could not serve as the sole source of protein. The minimum amount of lysine necessary to support growth in white rats is 1 per cent of the total protein; the minimum amount of tryptophan necessary for maintenance and growth in rats is 0.2 per cent. We considered these figures applicable to white mice in these experiments, as a fair margin was used.

The following diet was devised to meet total nutritive requirements: protein, 62.5 gm., consisting of purified zein, 35 gm.; 27.5 gm. in the form of wheat flour, potato, fruit and vegetables of the 3 per cent carbohydrate group containing 1 per cent protein. This amount of protein contained a total of 0.53 gm. of lysine; 0.374 gm. of tryptophan. The amounts of lysine and tryptophan were arrived at by calculations from tables published in *Biochemistry of Amino Acids* by Mitchell and Hamilton. Thus the lysine represents 0.85 per cent of the total P, or less than the 1 per cent minimum necessary to promote growth; the tryptophan is 0.6 per cent of the total protein; therefore, 3 times the 0.2 per cent minimum required for maintenance. The total calories were 3,214, the carbohydrates being supplemented by starch, the fats with butterfat freed of protein and crisco. The zein was made palatable by being made into cookies with wheat flour, starch, butter, and crisco. Details of the diet are given in the next section. The protein content of the diet mixture was determined by Kjeldahl analysis of an aliquot portion of the mixture.

The exact amount of each constituent was weighed and consumed daily. The first subject (the author) was a healthy male weighing 80 kg. For 3 days preceding the recorded period on the above diet he partook of a diet with approximately the same number

of calories but containing no zein and only 12 gm. of protein. The object was to allow for elimination of any stored amino acids that might affect the nitrogen balance in the subsequent experimental period. The data are recorded in Table I.

It will be noted that there are three periods in this experiment. Nitrogen equilibrium was maintained during the first period when the diet contained 35 gm. of zein and a total of 62.5 gm. of protein. During the two subsequent periods the total protein was reduced to 50 gm. and to 56.2 gm. respectively. In each of these two periods, nitrogen equilibrium was not maintained.

As a control to this experiment a healthy female subject, BKK, weight 53.6 kg., was placed on a similar diet with modification in proportion to caloric requirements. This diet contained 2,000 calories; 40 gm. of protein (22 gm. of zein). As may be seen in Table II, nitrogen balance was maintained on this diet.

Table I: Nitrogen Balance with Diet Having Low Lysine Content

RAK ht. 174 cm.

| Date, Dec. | Body weight, kg. | Diet, cal. | Cal. per kg. | Lysine in diet, gm. | Trypto-phan in diet, gm. | Zein in diet, gm. | Total pro-tein, gm. | Total N diet, gm. | Urine Vol., cc. | Urine Sp. gr. | Total urine N, gm. | Feces, N, gm. | N Bal. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 80.4 | 3,214 | 40 | .53 | .374 | 35 | 62.5 | 10.0 | 1,235 | 1,020 | 8.95 | 2.47 | —1.42 |
| 4 | 80.9 | 3,214 | 40 | .53 | .374 | 35 | 62.5 | 10.0 | 1,103 | 1,022 | 6.97 | 2.47 | + .58 |
| 5 | 81.2 | 3,214 | 40 | .53 | .374 | 35 | 62.5 | 10.0 | 650 | 1,035 | 5.90 | 2.47 | +1.63 |
| | | | | | | | | | | | Average 7.27 | | |
| 6 | 81.1 | 2,494 | 31 | .38 | .35 | 26.6 | 50 | 8.0 | 920 | 1,028 | 8.28 | 2.58 | —2.86 |
| 7 | 80.6 | 2,494 | 31 | .38 | .35 | 26.6 | 50 | 8.0 | 830 | 1,024 | 6.87 | 2.58 | —1.45 |
| 8 | 80.6 | 2,494 | 31 | .38 | .35 | 26.6 | 50 | 8.0 | 1,010 | 1,023 | 7.57 | 2.58 | —2.15 |
| 9 | 80.5 | 2,494 | 31 | .38 | .35 | 26.6 | 50 | 8.0 | 1,100 | 1,019 | 7.05 | 2.58 | —2.63 |
| | | | | | | | | | | | Average 7.44 | | |
| 10 | 80.4 | 2,854 | 35 | .46 | .36 | 30.8 | 56.2 | 9.0 | 580 | 1,035 | 7.49 | 2.20 | — .69 |
| 11 | 80.2 | 2,854 | 35 | .46 | .36 | 30.8 | 56.2 | 9.0 | 650 | 1,032 | 8.01 | 2.20 | —1.21 |
| 12 | 80.2 | 2,854 | 35 | .46 | .36 | 30.8 | 56.2 | 9.0 | 545 | 1,038 | 7.25 | 2.20 | — .45 |
| | | | | | | | | | | | Average 7.58 | | |

Table II: Nitrogen Balance with Diet Having Low Lysine Content

BBK ht. 158 cm.

| Date, Dec. | Body weight, kg. | Diet, cal. | Cal. per kg. | Lysine in diet, gm. | Trypto-phan in diet, gm. | Zein in diet, gm. | Total pro-tein, gm. | Total N diet, gm. | Urine Vol., cc. | Urine Sp. gr. | Total urine N, gm. | Feces, N, gm. | N Bal. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 53.6 | 2,000 | 37 | 0.33 | 0.22 | 22.0 | 40 | 6.52 | 925 | 1,020 | 4.59 | .91 | +0.09 |
| 6 | 53.0 | 2,000 | 37 | 0.33 | 0.22 | 22.0 | 40 | 6.52 | 870 | 1,019 | 5.90 | .91 | +0.09 |
| 7 | 52.8 | 2,000 | 37 | 0.33 | 0.22 | 22.0 | 40 | 6.52 | 600 | 1,024 | 5.54 | .91 | +0.09 |
| | | | | | | | | | | | | Average 5.52 | |
| 8 | 52.8 | 2,000 | 37 | 0.33 | 0.22 | 22.0 | 40 | 6.52 | 385 | 1,030 | 5.21 | .91 | +0.09 |
| 9 | 52.9 | 2,000 | 37 | 0.33 | 0.22 | 22.0 | 40 | 6.52 | 580 | 1,026 | 5.94 | .91 | +0.09 |
| 10 | 52.9 | 2,000 | 37 | 0.33 | 0.22 | 22.0 | 40 | 6.52 | 850 | 1,020 | 5.93 | .91 | +0.09 |

## PART II

### Animal Experiments

When it had been found that nitrogen equilibrium in the human subject can be maintained on a palatable and inexpensive diet lacking in the essential growth substance, lysine, it remained to be determined whether a similar diet would be effective in retarding the growth of malignant tumors in mice.

For these experiments the No. 3 Marsh strain of mice was used, obtained through the kindness of Dr. B. T. Simpson, of the State Institute for the Study of Malignant Diseases, Buffalo, N. Y., to whom grateful acknowledgment is made for this stock. Twelve mice of this strain were obtained, which formed the nucleus for our colony. This strain shows a high incidence of spontaneous mammary carcinoma, exceeding 90 per cent in the females.

This original colony was kept for some time on a stock diet consisting of 30 per cent whole milk powder and 70 per cent whole wheat flour, supplemented by 3 per cent sodium chloride of the mixture, 3 per cent cod liver oil, and 0.13 per cent iron citrate. The mice remained in good condition on this diet and reproduced freely. Tumors appeared in most of the females from the age of 5 months on. The rate of growth of the tumors was determined by measurement of two dimensions of the tumor in millimeters. The product of these two dimensions was taken as the measure of

growth. Measurements were taken twice a week and plotted against time. The mice were weighed twice a week. On the stock diet, food was given *ad libitum* but on the special diets the amounts consumed were weighed daily.

| Lysine-deficient diet fed to mice | Grams | Protein, gm. | Lysine, gm. | Tryptophan, gm. |
|---|---|---|---|---|
| Zein | 12 | 12 | 0 | 0.02 |
| Ground whole wheat | 50 | 5 | 0.047 | 0.05 |
| Potato (raw) | 10 | 0.3 | 0.012 | 0.006 |
| Tomato juice | 25 | 0.25 | 0.017 | 0.0025 |
| Cod liver oil | 3 | 0 | 0 | 0 |
| Crisco | 20 | 0 | 0 | 0 |
| B complex | 0.1 | 0 | 0 | 0 |
| Salt (NaCl) | 4 | 0 | 0 | 0 |
| (Iron citrate) | | | | |
| | | 17.55 | 0.076 | 0.0785 |

The lysine content equals 0.4 per cent of total protein.

The tryptophan content equals 0.45 per cent of the total protein. (Amount necessary for growth: lysine, 1 per cent of the total protein; tryptophan, 0.2 per cent.) Hence this diet is deficient in necessary lysine for growth of normal animals but adequate in tryptophan. The B complex used was a standardized concentrate containing 50 USP units of $B_1$; 0.05 mgm. riboflavin, in addition to equivalent units of the other members of the B complex

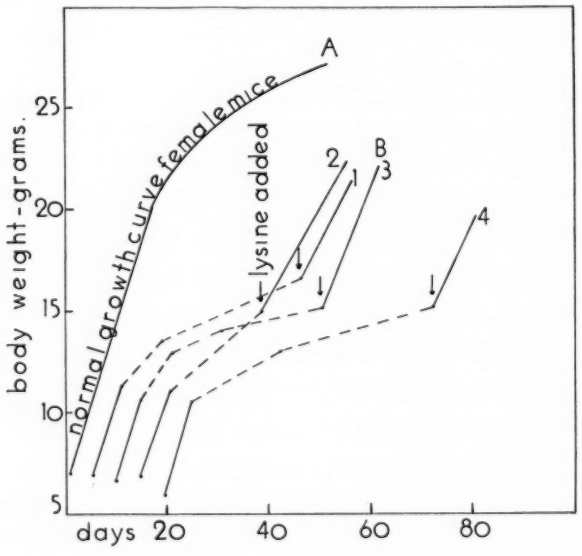


FIG. 1.—Growth of young female mice. Stunting of growth on lysine-deficient diet and resumption of normal growth when lysine was added. First leg of curve B shows growth on stock diet. Broken line shows stunting effect on lysine-deficient diet, followed by acceleration when lysine was added.

Autopsies were done on some of the animals, all of which proved to have adenocarcinomas, the variety of neoplasm to which this strain is predisposed.

*Young mice without tumors.*—Twelve healthy young female mice were placed on our stock diet and their growth curve was plotted (Fig. 1, A). The growth inhibiting effect of the lysine diet shown at B, broken line, and the accelerating effect of adding 0.6 per cent pure crystalline natural *l* (+) lysine is also apparent. The dihydrochloride of lysine was used.

*Tumor growth on lysine-deficient diet.*—Female mice showing spontaneous mammary carcinomas were put into individual cages and fed on the lysine-deficient diet for a sufficient time to show the inhibiting effect of this diet on the growth of the tumors. Six-tenths per cent lysine was then added, and this produced a distinct acceleration of growth (Fig. 2). It was noted that in all cases where the deficient diet was started after the tumors had reached considerable size the

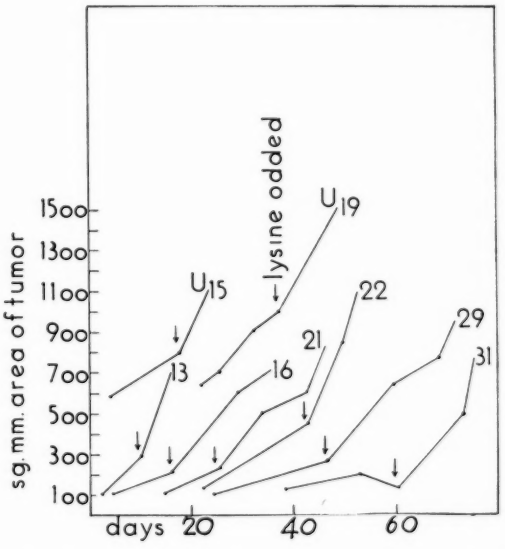


FIG. 2.—Tumor growth. Retardation on lysine-deficient diet during period of 10 to 30 days, and acceleration of growth when lysine is added (Arrow).

(U = ulcerated.)

retarding effect was less than in those where this diet was fed when the tumors were small. This is well illustrated in the chart in mice 15 and 19. Because of this observation, experiments were carried out in which the deficient diets were begun after tumors had reached considerable size and continued over a considerable period or until tumors either ulcerated or the animals died (Fig. 3, A). It is apparent that the retarding effect of the diet was less in C than in A, evidently because of the large size of the tumors. Furthermore, the retardation was of short duration, followed by a spontaneous acceleration of growth corresponding to the rate in tumors in animals fed on the stock diet. Curve B illustrates the result when the lysine deficient diet was fed from the time tumors first became measurable and continued until the tumors ulcerated or the animal either died or began losing weight.

From the data presented in Tables III, IV, and V it is apparent that the acceleration in rate of tumor

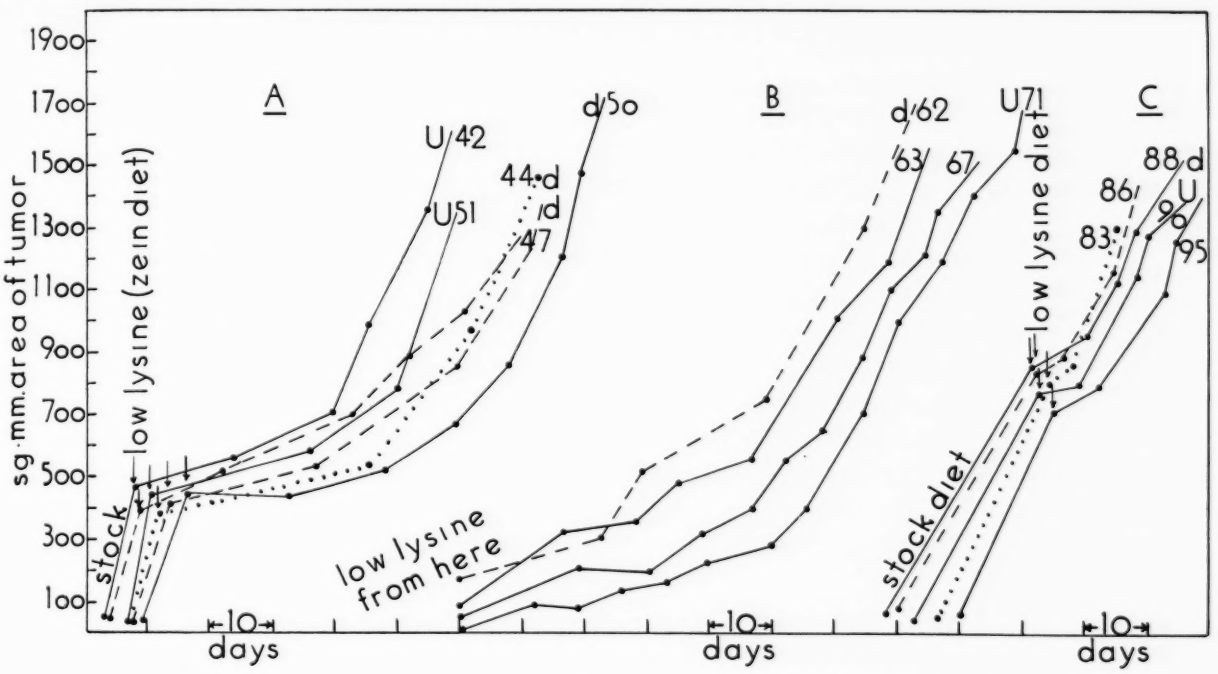


FIG. 3.—Tumor growth. Retardation for period of 30 to 50 days but failure to retard growth in later stages. A = Female mice with tumors kept on stock diet until tumors reached 300 to 400 sq. mm. Then lysine-deficient diet given. Tumor growth was retarded for first 30 to 50 days then became accelerated with no change in diet. B = Low lysine diet begun as soon as measurable tumors appeared. Growth was slow for 40 to 60 days then became rapid. C = Stock diet given until tumors reached size of 700 to 800 sq. mm. Then lysine-deficient diet given. From this point there was a short retarding effect followed by acceleration of growth.

(U = ulceration. D = diet.)

TABLE III: AVERAGE DAILY FOOD CONSUMPTION OF THE YOUNG MICE WHOSE GROWTH CURVES ARE SHOWN IN B, FIG. 1

| Mouse No. | On zein diet, gm. | Lysine added, gm. |
|---|---|---|
| 1 | 2.08 | 2.22 |
| 2 | 2.16 | 2.19 |
| 3 | 2.31 | 2.35 |
| 4 | 2.27 | 2.24 |

TABLE IV: AVERAGE WEIGHTS AND AVERAGE DAILY FOOD CONSUMPTION CALCULATED FOR 25 GM. OF BODY WEIGHT OF MICE WHOSE RATE OF TUMOR GROWTH IS PLOTTED IN FIG. 2

| | On basic lysine-deficient diet | | On basic diet plus lysine | |
|---|---|---|---|---|
| Mouse No. | Average weight, gm. | Average food consumption, gm. | Average weight, gm. | Average food consumption, gm. |
| 13 | 26.2 | 2.43 | 25.9 | 3.02 |
| 15 | 30.4 | 2.98 | 30.2 | 2.84 |
| 16 | 29.9 | 2.87 | 28.8 | 2.74 |
| 19 | 27.5 | 2.62 | 28.2 | 2.86 |
| 21 | 31.1 | 3.02 | 30.6 | 2.92 |
| 22 | 26.7 | 2.9 | 27.1 | 3.03 |
| 29 | 28.8 | 2.68 | 28.5 | 2.70 |
| 31 | 26.6 | 2.48 | 27.7 | 2.66 |

TABLE V: AVERAGE WEIGHTS AND THE AVERAGE DAILY FOOD CONSUMPTION CALCULATED PER 25 GM. OF BODY WEIGHT OF MICE WHOSE RATE OF TUMOR GROWTH IS PLOTTED IN FIG. 3, A, B, AND C

| | On stock diet | | On basic diet (Low lysine) | | Late period tumors, 1,200 sq. mm. or more | |
|---|---|---|---|---|---|---|
| Mouse No. | Average weight, gm. | Average food consumption, gm. | Average weight, gm. | Average food consumption, gm. | Average weight, gm. | Average food consumption, gm. |
| 42 | 25.8 | 3.04 | 27.2 | 2.96 | | |
| 44 | 29.2 | 3.42 | 31.6 | 3.34 | | |
| 47 | 28.4 | 2.82 | 27.9 | 3.04 | | |
| 50 | 27.0 | 2.90 | 27.4 | 2.96 | | |
| 51 | 26.6 | 3.16 | 28.2 | 3.22 | | |
| 62 | 31.2 | 3.33 | 30.8 | 3.10 | | |
| 63 | 26.9 | 2.74 | 27.0 | 2.88 | | |
| 67 | 29.2 | 3.12 | 29.8 | 3.22 | | |
| 71 | 27.4 | 2.88 | 27.4 | 2.94 | | |
| 83 | 29.3 | 2.96 | 29.1 | 2.84 | | |
| 86 | 25.5 | 2.77 | 27.2 | 2.66 | | |
| 88 | 28.5 | 2.68 | 26.8 | 2.59 | | |
| 90 | 30.1 | 2.92 | 29.9 | 2.78 | | |
| 95 | 27.8 | 3.06 | 28.2 | 2.69 | | |
| 42 | — | — | — | — | 23.8 | 2.40 |
| 50 | — | — | — | — | 25.5 | 2.63 |
| 63 | — | — | — | — | 24.7 | 2.82 |
| 71 | — | — | — | — | 23.9 | 2.38 |
| 86 | — | — | — | — | 22.7 | 2.57 |
| 88 | — | — | — | — | 24.2 | 2.91 |

growth is not due to increased food consumption. The body weight of the animals remained fairly constant when the animals were fed on the stock diet as also during the period when the lysine-deficient diet was given, and during the period when the basic diet plus lysine was consumed. The loss of weight in the late periods of tumor growth shown in Table V, last two columns, is partly attributable to decreased food consumption. However, the rate of tumor growth continued at a rapid rate, as shown in Fig. 3, despite the loss of body weight during the late period.

## DISCUSSION

The data from these experiments clearly show that the rate of tumor growth in mice can be inhibited for short periods of 20 to 60 days when these animals are fed on a diet in which the amino acid lysine is deficient. This is further substantiated by the rapid increase in rate of growth of the tumor when lysine is added to the diet previously deficient in this amino acid. These results are in agreement with the work of Voegtlin and his associates (7, 8).

However, it is apparent from the present experiments that when the lysine-deficient diet is continued for a longer period than 30 to 60 days the retarding effect gives way to a resumption of rapid growth, equal in rate to that on a stock diet of adequate lysine content. It is also apparent that when the lysine-deficient diet is begun after the tumors have reached considerable size the retarding effect is absent or very slight and of brief duration.

On the basis of these observations it would seem that neoplastic tissue, like normal somatic tissue, requires lysine for growth. In the early stages of growth, when the tumor is deprived of this amino acid, growth is retarded, whereas later on a mechanism is acquired by which either lysine is obtained independently of the diet, or growth is able to continue without lysine. Just what this mechanism may be is open to speculation. Lysine may be obtained from autolysis or necrosis of tumor tissue; from protein reserves of the body; or from breakdown of body tissues from the toxic action of products of the malignant tumor.

The latter source seems likely, because of the loss of weight in the animals during the late stages of tumor growth. Despite this loss of total body weight, the tumors continued the same rapid rate of growth.

## CONCLUSIONS

The rate of normal growth in mice, and the rate of growth of spontaneous mammary carcinoma, can be retarded for short periods by a diet deficient in the amino acid lysine. Growth may be resumed at a normal rate upon the addition of lysine, which would indicate that this amino acid is essential for both normal and malignant growth.

When a lysine-deficient diet is fed to tumor mice for a considerable period the inhibiting effect on growth wears off and the neoplasm resumes rapid growth. If the lysine-deficient diet is begun when the tumors have reached an advanced stage of growth the inhibiting effect is either not apparent or very short.

Although the diets deficient in lysine were found suitable for human consumption, if the findings for tumor-bearing mice should obtain in man these diets would not be expected to offer any promise as a therapeutic measure.

## REFERENCES


1. Courrier, R., and Coste, G. Cancer et acides aminés indispensables a la croissance. Compt. rend. Soc. de Biol., **115**:631. 1934.
2. Kocher, R. A. The Hexone Bases of Malignant Tumors. J. Biol. Chem., **22**:295-303. 1915.
3. Osborne, T. B., and Mendel, L. B. Amino-Acids in Nutrition and Growth. J. Biol. Chem., **17**:325-349. 1914.
   Osborne, T. B., and Mendel, L. B. The Amino-Acid Minimum for Maintenance and Growth as Exemplified by Further Experiments with Lysine and Tryptophane. J. Biol. Chem., **25**:1-12. 1916.
4. Rose, W. C. Nutritive Significance of Amino Acids. Physiol. Rev., **18**:109-136. 1938.
5. University of California Bulletin, 3rd Series X, No. 6, 1916, p. 125.
6. Voegtlin, C., Johnson, J. M., and Thompson, J. W. Glutathione and Malignant Growth. Pub. Health Rep., **51**: 1689-1697. 1936.
7. Voegtlin, C., and Maver, M. E. Lysine and Malignant Growth. Effect on Malignant Growth of Gliadin Diet. Pub. Health Rep., **51**:1436-1444. 1936.
8. Voegtlin, C., and Thompson, J. W. Lysine and Malignant Growth. Amino Acid Lysine as Factor Controlling Growth Rate of Typical Neoplasm. Pub. Health Rep., **51**:1429-1436. 1936.

# Characterization of an Influence Affecting Growth of Transplantable Leukemias in Mice*

L. W. Law, Ph.D.

*(From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.)*

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It has been shown in previous communications (1, 2) that an influence transmitted in the milk of certain lactating female mice was effective in influencing the growth of lymphoid leukemias (lines LL449 and and P1543) in mice normally refractory to their growth. This "susceptibility influence" was found to be present during the first 10 days of the lactation period. The influence was found to be transmitted to second generation mice obtained by mating litter mates of refractory foster-nursed mice. Total deprivation of "susceptible" milk in mice of the susceptible strain did not affect the response of these mice to inoculations of leukemic cells. A change in the growth capacity of the malignant cells of lymphoid leukemias, lines LL449 and P1534, whereby leukemic cells could be grown by serial transfer in totally refractory hosts was observed. This change occurred following initial growth of the malignant cells in refractory mice that were foster-nursed by mothers of the susceptible strain. Such serial transfers have subsequently been carried through 10 generations. These data suggested that the type of reaction provoked in refractory foster-nursed mice depended upon the quantity of "susceptibility influence" obtained.

In an attempt to learn if other leukemias as well as other types of tumors were affected by the same or similar influences a total of 14 transplantable malignant tumors were tested (Table I). Previous tests have been made on a few transplantable leukemias (1, 2). Malignant tumors arising in one inbred strain were inoculated into other inbred strains. The inbred strains of mice used throughout were A, C, L, C3H, C57 black, C57 brown, and dba. Several sublines of some of the inbred strains were used, particularly within the dba and C57 black strains. At least 50 refractory control mice and 50 refractory foster-nursed mice were inoculated with each tumor type. Refractory mice were foster-nursed by lactating mothers of the strain of origin of the malignant process, as described in a previous communication (1). The greatest percentage of takes occurred in foster-nursed mice of sublines of the strain of origin of the neoplasm but was not limited to these.[1]

Malignant tumors induced by carcinogens as well as those arising spontaneously were used. Transplantable tumors carried by serial transfer for many generations and tumors of recent origin also were tested.

Myeloid leukemia, line C1498, was found to grow

TABLE I: TYPES OF MALIGNANT TUMORS TESTED FOR PRESENCE OF "SUSCEPTIBILITY INFLUENCE"

| Tumor number | Tumor type | Generation of transplant | Strain of origin | Effect of foster nursing |
|---|---|---|---|---|
| P1534 (S) * | Lymphosarcoma | 10 | dba | + |
| LL493 (I) * | Myeloid leukemia | 5 | " | — |
| LL449 (I) | Lymphoid " | 6 | " | + |
| C1498 (S) | Myeloid " | 13 | C57 black | + |
| S91 (S) | Melanoma | 40+ | dba | — |
| LL552 (I) | Fibrosarcoma | 5 | " | — |
| C252 (S) | " | 40+ | C57 leaden | — |
| P1591 (S) | Myeloid leukemia | 10 | dba | — |
| P1592 (S) | Lymphoid " | 10 | " | — |
| LL336 (I) | Monocytic " | 5 | " | — |
| LL593 (I) | Lymphoid " | 10 | " | + (possible) † |
| dbrB (S) | Mammary adenocarcinoma | 500+ | " | — |
| P1682 (S) | Lymphoid leukemia | 12 | " | — |
| P1679 (S) | " " | 9 | " | + (possible) † |

* (S)=spontaneous tumor; (I)=tumor induced by carcinogen.
† = See text.

[1] Pronounced physiological differences are found to exist between sublines within inbred strains of mice particularly within the dba and C57 black strains.

* Aided by a grant from the Commonwealth Fund.

in refractory hosts following foster nursing of refractory mice by lactating females of the susceptible strain.

The growth capacities of two other leukemias, lines LL593 and P1679, both lymphoid in origin, were possibly affected in a similar manner by an influence transmitted through the milk of susceptible mice. The numbers of experimental mice used here were smaller than in other series. Leukemia, line P1679, produced 100 per cent takes in subline 2 of the dba strain (subline of origin) and failed to grow in 50 subline 1 mice. On the other hand 35 per cent takes resulted following inoculations in dba, subline 1 mice foster nursed by dba subline 2 mothers (35 mice). Leukemia, line LL593, grew only in dba, subline 1 mice and failed completely to grow in mice of subline 2. Foster nursing of subline 2 mice by subline 1 mothers produced 25 per cent susceptibility (25 mice). Mice of other inbred strains, A, C, L, C3H, C57 black and C57 brown foster nursed by susceptibile mothers remained refractory to inoculations of both lines of leukemia, LL593 and P1679.

No such influences transmitted in the milk were found to affect the growth capacities of 2 fibrosarcomas (line LL552 and line C252), a melanoma (line S91), a mammary adenocarcinoma (line dbrB), a monocytic leukemia (line LL336), 2 lymphoid leukemias (line P1682 and line P1592) and 2 myeloid leukemias (line P1591 and line LL493).

Leukemia C1498 arose in a female mouse, 10 months of age, of the B subline, C57 black inbred strain. The liver was greatly enlarged, gray, friable, but not nodular. The spleen was enlarged, pale, and nodular. All lymph nodes were found negative upon gross examination. Ascites was present. No blood count was obtained on this animal. The first 7 generations of serial transplant in B subline mice were carried by Dr. A. M. Cloudman, of this laboratory. This leukemia has now been carried through 25 transplant generations and shows the same typical growth characteristics.

Following the subcutaneous inoculation of leukemic cells from the liver or spleen a definite growth appears at the site of inoculation in 1 or 2 weeks. The liver and spleen show pronounced infiltration of cells myeloid in origin. In the gross these organs are extremely swollen, friable, marked with huge grayish nodules, and contain numerous hemorrhagic areas. There occurs at first an infiltration in the mesenteric lymph nodes, followed usually by infiltration in the lumbar and renal nodes and frequently by infiltration in the subcutaneous nodes. Considerable invasion has been seen in the lungs, kidneys, adrenals, and ovaries. Peripheral white blood cell counts were from 20,000 to 150,000 with approximately 25 per cent of the cells myeloid in origin, either myeloblasts or myelocytes, which give a positive peroxidase reaction.

Transplants of leukemic tissue into mice of inbred strains dba, C57 brown, A, C, L, C3H, and C and other sublines of C57 black failed to grow. Following foster nursing, however, some refractory mice of the C57 brown and dba strains showed a local growth of leukemic cells of leukemia, line C1498, with a subsequent regression in most cases. Mice of subline W of the C57 black strain that were refractory (3.5 per cent positive) proved to be 66.7 per cent susceptible following foster nursing by mothers of the B subline, C57 black strain (Table II). Sixteen of the 44 mice showed only a local, but progressive, subcutaneous growth of leukemic tissue, followed in late stages by invasion into surrounding subcutaneous lymph nodes and a moderate escape of myeloid cells into the blood stream. Regressions were infrequent. Most foster-nursed W subline mice developed the typical systemic leukemia.

The susceptibility influence has been found to be transferred by B subline mothers to suckling young of the W subline as late as the 18th day of lactation, indicating the presence of the influence throughout the lactation period.

Following transfer of the susceptibility influence to refractory mice by foster nursing, this influence was maintained in normally refractory mice by breeding. Three generations of W subline mice grew leukemic tissue of line C1498 myeloid leukemia without resort to subsequent foster nursing by B subline mothers.

In an attempt to identify this influence transferred through the milk of susceptible mothers, various tissue extracts were administered to young refractory mice by both feeding and inoculation. Leukemia, line C1498, was used. The data obtained are only preliminary because of interruption of this work.

It was found that liver, spleen, or mammary gland, homogenized and extracted with physiological saline, when fed to or inoculated into refractory mice from 10 days to 3 weeks of age resulted in the development of a susceptibility to growth of the leukemic cells of line C1498. The most potent extract was obtained from lactating mammary glands (Table III).

In order to standardize the test procedure 0.25 gram equivalent of tissue in physiological saline (0.25 cc.) was used throughout all tests. Refractory mice 3 weeks of age were used as test animals and the inoculations of tissue extract were made subcutaneously into the right axillary region. A single inoculation of tissue extract was given each test animal. In all cases 7 days following the inoculation of homogenized tissue extract the refractory mice were inoculated with leukemic cells of myeloid leukemia, line C1498. Transfer

of the influence by inoculation of 0.25 gram equivalent homogenized mammary gland extract resulted in 17 of 34 refractory subline W mice growing the leukemic cells of line C1498. In contrast, only 23.1 per cent W subline mice grew the leukemia following inoculations with homogenized splenic extract of male or female susceptible mice.

A series of tests were made using as a source of susceptibility influence mammary gland extract. Each separate test was controlled by uninoculated subline W mice, which in most cases were litter mates to animals showed complete systemic growth of leukemic cells, a certain heat stability is indicated.

4. The Seitz filter failed to remove all the susceptibility influence in mammary gland extract. Two separate tests were made and in each the filtered extract produced susceptibility to transplanted leukemic cells.

5. Desiccation of mammary gland and of leukemic tissue was accomplished under vacuum for 4 hours at room temperature. Extracts of desiccated mammary gland produced susceptibility in only 1 of 10

TABLE II: REACTIONS OF NORMAL AND FOSTER-NURSED MICE TO TRANSPLANTABLE MYELOID LEUKEMIA, LINE C1498

| Donor of leukemia cells | Recipient | Number of mice | Number positive * | Per cent positive |
|---|---|---|---|---|
| C57 black, B subline | C57 black, B subline | 131 | 130+++; 1— | 99.2 |
| " " | C57 " , W " | 86 | 1+++; 3+; 82— | 3.5 |
| " " | C57 " , W " fostered by C57 black, B subline | 66 | 28+++; 16+; 22— | 66.7 |

* (+++)=systemic infiltration of leukemic cells; (+)=local progressive growth of leukemic cells; (—)=no growth.

TABLE III: REACTIONS OF REFRACTORY MICE (C57 BLACK, W SUBLINE) TO TISSUE EXTRACTS

| Material inoculated | Number of mice | Number positive * | Per cent positive reaction |
|---|---|---|---|
| Mammary gland extract (0.25 gm. equiv.) | 34 | 15+++; 2+ | 50.0 |
| Splenic extract | 13 | 3+++ | 23.1 |
| Desiccated extract (C1498 leukemic tissue) | 20 | 2+++; 3+ | 25.0 |
| 40 hour dialysate | 17 | 8+++ | 47.0 |
| " " non-dialysate | 13 | 7+++ | 53.8 |
| Glycerolated mammary gland | 21 | 13+++; 3+ | 76.2 |
| Heated mammary gland extract | 25 | 4+++; 1+ | 20.0 |
| Desiccated extract (mammary gland) | 10 | 1+++ | 10.0 |
| Partially digested milk (gastric content) | 10 | 7+++ | 70.0 |
| Seitz filtrate | 6 | 2+++ | 33.3 |
| Controls † | 86 | 1+++; 3+ | 3.5 |

* (+++)=systemic infiltration of leukemic cells; (+)=local progressive growth leukemic cells.
† Litter mates to mice used in experimental series.

the experimental group. The following observations were made:

1. Dialysis of mammary gland extract against physiological saline was accomplished by use of parchment paper (Will Corporation—Grade A). Dialysis was carried on for 40 hours at −4° C. Refractory test animals were rendered susceptible by both the dialysate and non-dialysate (Table III). Five separate tests were made for each of the extracts.

2. Homogenized mammary gland, stored in 50 per cent glycerol for 30 days at −4° C, then extracted with physiological saline, proved to contain the susceptibility influence. Four separate series of tests were made and, in total, 16 of 21 inoculated mice proved susceptible to transplanted leukemic cells.

3. Mammary gland extract heated for 20 minutes at 85° C in a water bath produced susceptibility in 5 of 25 animals. One animal in each of 5 separate tests proved susceptible. Since 4 out of 5 experimental mice. On the other hand, extracts of desiccated leukemic cells produced susceptibility in 5 of 20 mice, with 1 susceptible animal in each of 5 test series.

6. In another attempt to obtain a source of material for characterization tests of the susceptibility influence, partially digested milk obtained from stomachs of subline B (susceptible) mice was extracted and inoculated into refractory subline W mice. Gastric contents were removed from suckling mice 1 hour after suckling. Seven of 10 mice proved susceptible to the growth of transplanted myeloid leukemia cells. As a control experiment milk obtained from stomachs of strain A and subline W, C57 black mice was extracted and inoculated into test mice. These mice remained refractory to inoculations of leukemic cells. It has been found, in contrast, that digestion or partial digestion for 30 minutes will inactivate the mammary tumor inciter.

## SUMMARY AND CONCLUSIONS

1. An influence transmitted in the milk of certain lactating female mice was found to be effective in promoting the growth of two transplantable lymphoid leukemias and a transplantable myeloid leukemia in normally refractory mice. Preliminary data indicate the probability that such an influence, or influences, affects the growth potentialities in refractory mice of two other lymphoid leukemias.

2. The growth in mice of refractory strains of 2 fibrosarcomas, a melanoma, a mammary adenocarcinoma, a monocytic leukemia, and 2 other lines of myeloid leukemia were found not to be affected by an influence or influences transmitted in the milk.

3. The susceptibility influence was found to be present throughout the period of lactation in susceptible lactating mice.

4. Following transfer of the susceptibility influence to refractory mice by foster nursing, this influence was found to be maintained through three generations, by breeding mice of the normally refractory strain.

5. Saline extracts prepared from homogenized liver, spleen, or mammary gland were found to contain the susceptibility influence of myeloid leukemia, line C1498.

6. The following characteristics of the susceptibility influence affecting the growth of this myeloid leukemia were found: (a) It will apparently dialyze through parchment paper at −4° C. Five separate tests were made and in each the dialysate contained the influence. (b) It remains stable in 50 per cent glycerol for 30 days at −4° C. (c) A certain heat stability is indicated. The extract heated for 20 minutes at 85° C. rendered refractory mice susceptible to the growth of leukemia cells. (d) The Seitz filter apparently failed to remove all the influence from an extract. (e) Desiccation in a vacuum for 4 hours at room temperature inactivated the influence present in mammary gland extract. Desiccation under similar conditions failed to inactivate completely the influence in leukemia cells. (f) Digestion apparently failed to inactivate the influence. Four separate tests were made and in each test refractory animals were rendered susceptible by partially digested milk. This does not conclusively prove that the influence is unaffected by digestion. Gastric contents obtained 1 hour after suckling contained the susceptibility influence. This is in contrast to the effect of digestion on the mammary tumor inciter.


## REFERENCES


1. Law, L. W., Foster Nursing and the Growth of Transplantable Leukemias in Mice. Cancer Research, **2**:108-115. 1942.
2. Law, L. W., The Effect of Foster Nursing on the Growth of a Transmissible Leukemia in Mice. Science, **93**:381-382. 1941.

# Abstracts

## Experimental Research, Animal Tumors

**Besteht ein Zusammenhang zwischen dem Benzpyrengehalt und der carcinogen Wirkung des Teers? [Is There a Correlation Between the Benzpyrene Content and the Carcinogenicity of Tar?]** MIESCHER, G., ALMASY, F., and ZEHENDER, F. [Universitätsklinik, Zürich] *Schweiz. med. Wchnschr.*, **71**:1002-1007. 1941.

Since the benzpyrene content of tar, as determined spectroscopically, ran parallel with its carcinogenic activity, the authors regard the presence of other carcinogenic agents in adequate concentration as improbable.—W. H. W.

**Induction of Pulmonary Tumors in Mice with Ethyl Carbamate (Urethane).** NETTLESHIP, A., and HENSHAW, P. S. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:309-319. 1943.

It was observed that 26 of 29 mice subjected to urethane anesthesia and roentgen-ray exposure of a skin fold showed multiple pulmonary tumors. By a series of experiments it was determined that the urethane was the responsible agent, and that the roentgen-ray exposure, the anesthesia, and possible impurities in the urethane could be ruled out as factors in the increased incidence of lung tumors. In this first report it appeared that some increase in numbers of tumors occurs even after a single treatment with urethane. Strain A mice were also subjected to 14 weekly intraperitoneal injections of urethane, and the incidence of lung tumors was increased. Urethane, therefore, can be considered a carcinogenic agent for the induction of pulmonary tumors in mice. Tumors were not observed elsewhere.

The authors also include descriptions and microphotographs of these tumors and associated pulmonary changes. Lesions preliminary to the development of neoplasia were not found.—T. B. D.

**The Localisation of Experimental Tumours in Scars and Healing Wounds.** PULLINGER, B. D. [Imperial Cancer Research Fund, London, England] *J. Path. & Bact.*, **55**: 301-309. 1943.

Twenty years ago Deelman found (a) that tumors in tarred mouse skin tended to arise at the site of a wound, and (b) that they arose at such sites earlier than elsewhere. Many later workers have carried out similar experiments, with varying results. The paper describes experiments upon Simpson mice in which pieces about 0.8 cm. in diameter were excised at the time (fifth month) when papillomas began to appear from skin to which either (1) 3,4-benzpyrene (0.3% in liquid paraffin) or (2) 5,9,10-trimethyl-1,2-benzanthracene (0.06% in acetone) had been applied. Deelman's statement (a) was confirmed in 22 out of 40 mice, and statement (b) was confirmed in 9 out of 11 mice in experiment 2.—E. L. K.

**The Carcinogenicity of *p*-Dimethylaminoazobenzene in Diets Containing Hydrogenated Coconut Oil.** MILLER, J. A., KLINE, B. E., RUSCH, H. P., and BAUMANN, C. A. [Univ. of Wisconsin, Madison, Wis.] *Cancer Research*, **4**:153-158. 1944.

When albino rats were fed 0.06% of *p*-dimethylaminoazobenzene for 4 months in a synthetic diet containing 5% corn oil, the incidence of hepatic tumors at 6 months ranged from 53 to 64%. However, when the corn oil was replaced by hydrogenated coconut oil, the tumor incidence never exceeded 8% while in most groups it was zero. This effect of the hydrogenated coconut oil persisted when the rats received 25$\mu$gm. of pyridoxine and/or 40 mgm. of ethyl linolate daily.

By means of an analytical method adequate for the quantitative determination of 1 $\mu$gm. of *p*-dimethylaminoazobenzene, it was demonstrated that the azo dye was very stable in the diets *in vitro*. Attempts to demonstrate differences in the stability of the dye in the digestive tract were unsuccessful. It therefore appeared that the differences in carcinogenicity observed in the two oils were due to changes within the animal itself.—Authors' abstract.

**Untersuchungen über Tumorproteine: der Gehalt der Benzpyrensarkome an Nucleoproteid-P. [Investigations on Tumor Proteins: The Nucleoprotein P Content of Benzpyrene Sarcoma.]** RONDONI, P. [Institut für allgemeine Pathologie der Universität, and Krebsinstitut zu Mailand] *Schweiz. med. Wchnschr.*, **71**:1364-1365. 1941.

Benzpyrene sarcomas in the subcutaneous tissue of the rat contain more than 5 times as much nucleoprotein phosphorus as the normal mother tissue.

The livers of the tumor rats contained between 2 and 3 times as much as those of normal rats.—W. H. W.

**Enzymatic Activity of Normal Adult, Regenerating, Fetal, and Neoplastic Hepatic Tissues of the Rat.** GREENSTEIN, J. P., and THOMPSON, J. W. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:271-274. 1943.

The activity of normal adult rat liver, regenerating rat liver, fetal rat liver, and transplanted rat hepatoma 31 with respect to 12 enzyme systems was studied. Normal adult and regenerating livers were found to be very similar in their enzyme activities, as were fetal liver and hepatoma, but there were marked differences between the 2 groups. Arginase, catalase, zanthine dehydrogenase, the urea synthetic systems, and cystine, cytochrome, and *d*-amino acid oxidases were lower in fetal liver and in hepatoma than in adult liver. Acid phosphatase was slightly, and alkaline phosphatase markedly, higher in fetal liver and in hepatoma than in adult

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liver. Ribonucleodepolymerase, thymonucleodepolymerase, and amylase were about the same in all 4 kinds of hepatic tissue.—H. Q. W.

**Range in Activity of Several Enzymes in Normal and Neoplastic Tissues of Mice.** GREENSTEIN, J. P., and THOMPSON, J. W. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:275-281. 1943.

The activity of arginase, catalase, zanthine dehydrogenase, acid and alkaline phosphatase, thymonucleodepolymerase and ribonucleodepolymerase was determined in a number of normal mouse tissues and mouse tumors. The normal tissues included liver, breast, lymph nodes, marrow, spleen, kidney, muscle, skin, lung, intestinal and gastric mucosa, thymus, and pancreas. The tumors included 4 strains of hepatoma, 2 spontaneous mammary lymphomas, and several types of carcinoma and sarcoma. For each of the enzymes the range of activity of the different normal tissues was very wide, although it was somewhat less so for thymonucleodepolymerase than for most of the other enzymes. Among the different tumors the range of activity of each enzyme was less than for the different normal tissues. The difference is probably due to the decrease in the specific functional activity which takes place in many tissues when they become neoplastic.—H. Q. W.

**Note on the Liver Catalase Activity of Pregnant Mice and of Mice Bearing Growing Embryonic Implants.** GREENSTEIN, J. P., and ANDERVONT, H. B. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:283-284. 1943.

Previous work has shown that the liver catalase activity of mice, rats, and human subjects bearing various neoplasms is depressed. In the present paper the authors show that there is no depression in the catalase activity of the livers of pregnant mice and of mice bearing growing embryonic implants.—H. Q. W.

**Ascorbic Acid Content of Tumors and Homologous Normal Tissues.** ROBERTSON, W. VAN B. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:321-328. 1943.

The ascorbic acid content of 22 tumors of rats and mice, and of comparable normal tissues was determined. It was found that the ascorbic acid concentration of the tumors ranged from 15 to 70 mgm. per 100 gm. of fresh tissue. The ascorbic acid content of the tumors was not related to that of the tissues of origin. No correlation between ascorbic acid concentration and rate of growth was evident. Neither age of host nor site of transplantation materially affected the ascorbic acid concentration in the tumor.—H. Q. W.

**Metabolic Studies in Patients with Cancer of the Gastrointestinal Tract. XI. Postoperative Hypoproteinemia and Relationship of Serum Protein Fall to Urinary Nitrogen Excretion.** ARIEL, I. M., ABELS, J. C., PACK, G. T., and RHOADS, C. P. [Memorial Hosp., New York, N. Y.] *Surg., Gynec. & Obst.*, **77**:16-20. 1943.

Observations were made postoperatively upon patients with gastrointestinal cancer and, as controls, upon patients with non-neoplastic disease of the gastrointestinal tract or with gynecologic disorders who had undergone intra-abdominal operations. It was found that cancer patients frequently developed a negative nitrogen balance, but so, too, did the control patients and to as great a degree. The serum proteins, however, fell to a lower level in the cancer patients, hence it seems possible that the protein stores may be less in patients with gastrointestinal cancer than in the control groups studied.—J. G. K.

**Metabolic Studies in Patients with Cancer of the Gastrointestinal Tract. XIII. The Effect of Glycine on the Urinary Excretion of Creatine and Creatinine, Especially by Patients with Cancer of the Gastrointestinal Tract.** ABELS, J. C., KUPEL, C. W., PACK, G. T., and RHOADS, C. P. [Memorial Hosp., New York, N. Y.] *Cancer Research*, **4**:145-148. 1944.

The urinary excretion of creatine and creatinine was increased following the intravenous administration of glycine to normal subjects and to patients with benign gastrointestinal disorders. It failed to increase in 21 of 25 patients with gastrointestinal cancer and hepatic cirrhosis who had been given comparable amounts of glycine. It is suggested that this failure in the patients with gastrointestinal cancer is due to their coexisting hepatic insufficiency. The simultaneous ingestion of choline, as a source of free methyl groups, by patients with gastrointestinal cancer or hepatic cirrhosis, also failed to increase the urinary output of creatine and creatinine.—Authors' abstract.

**The Adrenals and Susceptibility to Transplanted Leukemia of Rats.** MURPHY, J. B., and STURM, E. [Rockefeller Inst., New York, N. Y.] *Science*, **98**:568. 1943.

A transplantable lymphatic leukemia that characteristically infiltrates the thymus extensively was inoculated into 2 groups of middle-aged rats. Normal rats of this age have atrophied thymus glands and are resistant to leukemia—only 46.9% of 32 inoculated animals developed the disease. The survival time in this group was 9.7 days. In rats adrenalectomized 15 days before inoculation the thymus is stimulated and susceptibility increased—90.3% of 31 rats developed leukemia. The average survival time of adrenalectomized animals following inoculation was 6.2 days.

In a second set of experiments adrenalectomy was shown to diminish the resistance induced in normally susceptible young rats by the injection of homologous defibrinated blood 2 weeks before inoculation with leukemic cells. With the blood injection alone, 33.9% of 59 inoculated rats developed leukemia. When rats were adrenalectomized before being injected with defibrinated blood, 76.8% of 43 inoculated animals developed leukemia. When they were adrenalectomized after the blood injection, 92.9% of 42 inoculated rats developed the disease. Control rats, and rats adrenalectomized but not treated otherwise, were 96.5 and 100% receptive respectively.—R. B.

**Comparative Antifibromatogenic Activity of Progesterone and Related Artificial Steroids.** LIPSCHÜTZ, A., BRUZZONE, S., and FUENZALIDA, F. [Nat. Health Service, Santiago, Chile] *Cancer Research*, **4**:179-185. 1944.

The preventive action of synthetic progesterone against abdominal fibroids elicited by α-estradiol in the guinea pig was obvious when only 13 to 24 μgm. of the 3-keto-steroid were absorbed daily from a subcutaneously implanted tablet.

The antifibromatogenic quantity of progesterone was less than the quantity of the fibromatogenic estrogen absorbed simultaneously.

There was still some preventive action with quantities of progesterone smaller than 13 μgm. daily (transitional zone).

On the contrary, quantities of pregnanedione or of allo-pregnanedione equal to antifibromatogenic quantities of synthetic progesterone, or quantities several times greater than the latter, were unable to prevent abdominal fibroids elicited by estradiol.

Since pregnanedione so far is not known to be progestational, our findings suggest that antifibromatogenic activity is concomitant to a certain extent with the progestational one.

This suggestion was corroborated by experiments with the nonprogestational Δ16-dehydroprogesterone, which revealed no antifibromatogenic activity even when quantities were absorbed that were 6 times greater than the antifibromatogenic threshold of progesterone.

When quantities were absorbed that were 10 to 30 times greater than the antifibromatogenic threshold of progesterone, the three nonprogestational steroids revealed some antifibromatogenic action comparable with that observed in the transitional zone with progesterone. This suggests that the three steroids might show progestational activity also if proper quantities were used.—Authors' summary.

**On the Antifibromatogenic Activity of Synthetic Progesterone in Experiments with the 17-Caprylic and Dipropionic Esters of α-Estradiol.** Lipschütz, A., and Grismali, J. [Nat. Health Service, Santiago, Chile] *Cancer Research,* **4**:186-190. 1944.

The fibromatogenic action of subcutaneously implanted tablets of the 17-caprylic ester and of the dipropionic ester of α-estradiol was prevented with quantities of progesterone not far from, or identical with, those that are necessary to antagonize the fibromatogenic action of free α-estradiol (antifibromatogenic threshold).

From these findings the conclusion can be drawn that esterification, though relatively protective against the inactivating action of the liver, does not confer protection against the antagonizing action of progesterone.

Our results suggest that the antagonistic action of this steroid against estradiol is effected not by interfering in the inactivation of estrogens in the liver but by rendering the reacting tissue unable to respond to the action of estrogens.—Authors' summary.

**Influence of Cross-Suckling on the Incidence of Mammary Cancer in High and Low-Cancer Strains of Mice.** Dmochowski, L., and Gye, W. E. [Imperial Cancer Research Fund, London, England] *Brit. J. Exper. Path.,* **24**: 223-226. 1943.

Experiments on two strains not hitherto used have confirmed the influence of the milk factor upon the incidence of mammary cancer. "Foster nursing of high-cancer strain RIII females by females of 'S' low-cancer strain reduced the incidence of mammary cancer in RIII females from 83 per cent at an average age of 8.5 months to 6.6 per cent at an average age of 9.3 months. Foster nursing of low-cancer strain 'S' females by females of RIII strain increased the incidence of mammary cancer in 'S' strain of mice from less than 1 per cent at an average age of 19 months to 29.6 per cent at an average age of 15.2 months."—E. L. K.

**Observations on the Inherited Susceptibility to Spontaneous Mammary Cancer in Mice.** Bittner, J. J. [Univ. of Minnesota Med. Sch., Minneapolis, Minn.] *Cancer Research,* **4**:159-167. 1944.

To determine the characteristics of the inherited susceptibility to spontaneous mammary cancer in mice, reciprocal matings were made between mice of the cancerous A and low cancerous C57 black (B) strains to produce mice of the $F_1$, $F_2$, and $F_3$ hybrid generations. The mice of the first generation either were nursed by their mothers or were fostered by females of the reciprocal stock to give 4 groups. The mice of each group were continued through the $F_3$ generation. Four groups of hybrids of the backcross generations to the low cancerous stock were also observed and were derived by mating the reciprocal $F_1$ and $F_2$ females to males of the B stock. All females were used as breeders to insure an adequate estrogenic stimulation of the mammary glands.

In this study the mice of the A stock were assumed to be homozygous for the inherited susceptibility for mammary cancer and the mice of the B strain were considered to be nonsusceptible.

No significant difference in the incidence of cancer was observed in the reciprocal hybrids that received the active mammary tumor milk agent, indicating that there was no intrauterine influence. Likewise, the females of the $F_1$ generations had approximately the same incidence as did the females of the cancerous A stock. The data obtained in the other hybrid generations either were in accord with the hypothesis that the inherited susceptibility was a single dominant, or more tumors were observed than would be expected according to this theory. In some generations the progeny of cancerous females had a higher incidence than did the progeny of noncancerous mothers.

Few spontaneous mammary tumors resulted in mice that were nursed by or descended from mothers nursed by females of the low cancerous strain.

The determination of the make-up of the inherited susceptibility was made more difficult in the hybrids because: (a) The incidence was influenced by the age at which the mothers developed spontaneous tumors. (b) The incidence was influenced by the litter in which the mice were born.

A considerable number of the mice of the nonsusceptible (low cancerous) strain developed mammary cancer after receiving the active milk agent. These observations indicate that some nonsusceptible mice may develop mammary cancer because of the inciting influence of the milk agent and the estrogenic stimulation. Thus all mammary tumors in mice do not result from the same causes.

Because there was no method of differentiating between mice that were somatically cancerous and those genetically cancerous, it was impossible to determine the number of factors transmitted, but it is probable that multiple factors were involved in the inherited susceptibility for spontaneous mammary cancer in mice.—Author's abstract.

**Karzinomstudien IV. Weitere Beiträge zur Frage der Unempfänglichkeit der Milz für Karzinom. [Further Observations on the Resistance of the Spleen to Carcinoma.]** KALLÓS, VON P., and KALLÓS-DEFFNER, L. *Schweiz. Ztschr. Path. u. Bakt.,* **3**:149. Abst. in *Schweiz. med. Wchnschr.,* **71**:944. 1941.

In rabbits unsuccessfully inoculated with the Brown-Pearce carcinoma the spleen was enlarged, though not so much as in those successfully inoculated. Histologically, however, there was no difference.

A future article will show that rabbits that resist grafts of this tumor produce a specific antibody against it, the presence of which can be demonstrated by complement-fixation.

White rabbits were less receptive to the tumor than pigmented ones, but their susceptibility could be considerably increased by splenectomy. Metastasis was more common than in the pigmented, and the daughter tumors were larger, especially in the kidney, and more malignant.

Two examples of metastases in the spleen are described which, unlike those in other organs, showed no tendency to infiltrate. There was a pronounced atrophy of the lymphoid-macrophage system in these spleens.—W. H. W.

**Tumor Antibodies (Brown-Pearce Carcinoma) in the Serum and in the Aqueous Fluid of the Anterior Chamber of the Eye.** APPEL, M., JANOTA, M., and STRAUSS, A. A. [Chicago, Ill.] *Proc. Inst. Med. Chic.,* **14**:108-110. 1942.

It was found that complement-fixing antibodies against the Brown-Pearce tumor were present in the blood serum but not in the aqueous humor of the eye in rabbits bearing this tumor or immune to it. Absence of antibodies is suggested as the reason for the successful growth of the Brown-Pearce tumor in the anterior chamber of the eye when it will not grow in the usual sites of transplantation. After puncture of the anterior chamber complement-fixing bodies were present possibly because of an alteration in permeability of the capillaries of the eye.—C. J. L.

**Serological Studies of Transplantable Mouse Tumor 15091a.** BUNTING, H. [Yale Univ. Sch. of Med., New Haven, Conn.] *Yale J. Biol. & Med.,* **15**:557-563. 1943.

Earlier histological and growth studies on the transplantable mouse tumor 15091a had suggested that regression followed the acquisition by the host of a resistance against the implanted tumor. In the present experiments no circulating complement-fixing antibodies against the tumor antigen could be demonstrated in sera of mice in which the tumor had regressed or had failed to grow after 2 or 3 implantations.

Injection of an aqueous extract of tumor 15091a into rabbits produced antibodies that reacted not only with the homologous antigen, but also with heterologous antigens from 5 spontaneous mammary carcinomas and 2 methylcholanthrene-induced sarcomas, irrespective of the strain of mice in which they arose. The reaction was weaker with 1 spontaneous carcinoma and 2 other induced sarcomas. No reaction was obtained with 2 spontaneous mammary carcinomas. The antiserum did not react with the majority of antigens prepared from normal mouse liver, kidney, brain, or mouse sera. These results are interpreted as indicating a similarity in antigenic composition between transplantable tumor 15091a and spontaneous and induced malignant tumors, but a difference between tumor 15091a and normal mouse tissue. The specifically reacting portion of the 15091a antigen was soluble in ether and alcohol.—J. L. M.

**Transplantable Benzpyrene-Induced Skin Carcinomata of Mice.** SALAMAN, M. H. [Strangeways Research Lab., Cambridge, England] *J. Path. & Bact.,* **55**:381-382. 1943.

Pieces from the deep surface of 15 epitheliomas induced in the skin of C57 mice by 3,4-benzpyrene in 280 days were covered with sulphapyridine and implanted sub cutem in 18 three months old mice of the same strain. Thirteen grafts from 10 mice were successful, and several of these had been carried on to the third, fourth, or fifth passage at the time of writing. "Histologically they are all malignant—squamous carcinomata. There are two main types, a highly anaplastic growth which progresses to central necrosis, and a keratinising growth in which the centre becomes horny. These types may co-exist, but one or the other is generally predominant." The characters of the primary and grafted tumors are shown well in 6 microphotographs. A similar experiment with an inbred strain of albino mice gave no successful grafts.—E. L. K.

**Chromophobe Adenoma-Like Lesions of the Rat Hypophysis. Frequency of the Spontaneous Lesions and Characteristics of Growth of Homologous Intraocular Transplants.** SAXTON, J. A., JR., and GRAHAM, J. B. [Cornell Univ. Med. Coll., New York, N. Y.] *Cancer Research,* **4**:168-175. 1944.

Chromophobe adenoma-like lesions of the hypophysis were found in 92 of 362 albino rats of the Yale strain. The frequency increased with advancing age, reaching 60% in male rats and 30% in female rats 600 and more days old. The lesions varied in size from microscopic nodules to masses weighing as much as 367 mgm. and were usually composed of cells with vacuolated chromophobic cytoplasm and oval nuclei containing coarse granules of chromatin.

Homologous intraocular transplants of adenoma-like tissue from two spontaneous lesions in male rats were carried into second and third serial intraocular generations. Transplants grew in 21 of 42 male recipients, but in none of 4 female recipients. Growth of transplants was extremely slow, and was often preceded by a latent period of several months. The growth rate was not accelerated in subsequent transfers, and the morphology did not change. Transplants grew as well in young as in old rats, and their fate was not influenced by the strain of the recipient. The presence of spontaneous adenoma-like lesions in the pituitaries of the hosts did not affect the behavior of transplants.

It is concluded that chromophobe adenoma-like lesions of the rat hypophysis are essentially neoplastic in character.—Authors' abstract.

**A Spontaneous, Transplantable, Adrenal Cortical Tumor Arising in a Strain C Mouse.** DALTON, A. J., EDWARDS, J. E., and ANDERVONT, H. B. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **4**:329-338. 1943.

Evidence is presented supporting the view that the tumor arose originally from relatively undifferentiated

cells of the outer adrenal cortex. Detailed pathologic and cytopathologic descriptions of material prepared with many staining technics are given, and numerous microphotographs are included. During transplantation the following changes developed: (1) rapid increase in growth rate, (2) more intense eosinophilic staining of cell cytoplasm, (3) prevalence of smaller cells with filamentous mitochondria in later generations, whereas large cells with spherical mitochondria were characteristic of the first two generations, and (4) disappearance of cells with lipid droplets in later generations. The transplanted tumors grew more rapidly in male animals, although the original tumor occurred in a female. No gross or histologic changes could be found in other organs of the host animals, except a focal edema that was noted in the deeper layers of the endometrial stroma of the uterus.—T. B. D.

**Carcinoma of the Adrenal Cortex in a Rabbit.** Hueper, W. C., and Ichniowski, C. T. [Warner Inst. for Therapeutic Research, New York, N. Y.] *Cancer Research,* **4**: 176-178. 1944.

A bilateral foam cell carcinoma of the adrenal cortex was found in a rabbit that 6 months previously had received an intravenous injection of the dye T-1824. The testes were completely atrophic.—Authors' abstract.

**Cellular Changes in Lymph Nodes of Experimental Mice with Special Reference to Plasma Cell Development.** Parsons, L. D. [The London Hosp., London, England] *J. Path. & Bact.,* **55**:397-407. 1943.

A continuation of the author's work on lymph nodes in mice in various conditions namely, (a) x-radiation, (b) treatment with Na 1,2,5,6-dibenzanthracene-9,10-endo $\alpha\beta$ succinate, (c) development of sarcomas induced by this compound or by methylcholanthrene, (d) grafting of sarcoma induced by methylcholanthrene, or of S 37, sub cutem or (e) intraperitoneally, and (f) injection of Na taurocholate sub cutem. Lymph nodes from such mice have now been examined after staining by the Unna-Pappenheim method, a normal node being stained at the same time as a guard against changes in the stain. Considerable plasma cell infiltration was found in the mesenteric nodes of only 3 out of 34 normal mice, and in 2 of these there were abscesses in the vicinity. In the groups named above the following conditions were found:

(a) Three to 10 days after irradiation (300 to 1,250 r) there is more or less complete replacement of lymphocytes by plasma cells.

(b), (c) Progressive increase in plasma cells amidst proliferated reticulum.

(d) Growth of these tumors is accompanied by abundant leukocytosis of myeloid type and by either myeloid or plasmacytoid change in the lymph nodes.

(e) Lymph nodes both within the peritoneal cavity and elsewhere show accumulation of plasma cells and proliferation of reticulum, and cells similar to plasma cells appear in the ascitic fluid. The changes in the mesenteric nodes begin within a few hours of grafting.

(f) In 16 mice lymphoid tissue was not markedly diminished; plasma cells were slightly increased but in only one case produced distinct infiltration. The injections caused considerable ulceration, but no tumors were produced.

Reticulosis is an early and constant feature of the changes observed and is coincident with the diminution of lymphocytes. No evidence was found of the development of plasma cells from lymphocytes. Proliferation of reticulum seems to start at the periphery and spread inwards to the medulla, and the plasma cells appear to develop in these areas. The appearances suggest that that plasma cells may be derived from the reticulum cells. The possible relationships between plasma cells and sarcoma cells are discussed. The paper is illustrated by 13 microphotographs.—E. L. K.

**Is the Action of Roentgen Rays Direct or Indirect? An Investigation of This Question by the Method of Human Marrow Culture.** Osgood, E. E. [Univ. of Oregon Med. Sch., Portland, Oregon] *Am. J. Roentgenol.,* **48**:214-219. 1942.

The author draws attention to the frequently noted observation that in cases of leukemia, Hodgkin's disease, and lymphosarcoma irradiation of lymph nodes in one area may be followed by a reduction in size of the spleen or nodes in nonirradiated areas. The object of the experiment reported was to determine whether or not this is the result of some substance transmitted by way of the blood stream.

Human marrow from which most of the non-nucleated erythrocytes had been removed was suspended in a cerebrospinal fluid-like medium and 2 samples irradiated with 400 r at 200 k.v. The suspensions were then centrifuged, and 4 combinations were prepared as follows: irradiated cells (1) in an irradiated medium, and (2) in a nonirradiated medium; nonirradiated cells (3) in an irradiated medium, and (4) in a nonirradiated medium. After a period of incubation cell counts were made.

The results showed no effect attributable to the presence of any substance in the medium from irradiated cells, and the only evidence of growth arrest was seen where the cells themselves had been irradiated.

The diminution of size of the nodes and spleen not irradiated is explained by the circulation of irradiated cells, and the author suggests that general body irradiation should be the method of choice in the treatment of these diseases—J. F.

**The New Nuclear Physics and Medicine.** Lawrence, J. H. [Univ. of California, Berkley, Calif.] *Am. J. Roentgenol.,* **48**:283-301. 1942.

The author presents an historical review of the development of artificial radioactivity and stresses its importance in biological and medical research and suggests the possibility of its future use in therapy. Radio-sulfur has been used as a tracer-substance in synthetic vitamin B. Radio-iron has been used in the study of absorption and utilization of iron in normal and anemic dogs. The use of radio-phosphorus in the treatment of leukemia is explained on the basis of the high uptake of phosphorus to form the nucleoprotein of the leukemic cells. Radio-iodine is shown to be of use in studying iodine metabolism and reference is made to its possible value in the treatment of hyperthyroidism. In carcinoma of the thyroid

it seems to be of no value since the cancer cells do not take up iodine as do the normal cells of the thyroid gland. Numerous radioactive elements are discussed and their usefulness explained. The elements rendered radioactive by the cyclotron have relatively short periods of radioactivity and so will do relatively little harm in the body and their presence and concentration can always be determined by the use of the Geiger counter and by photography.

The production, physical and biological effects of the neutron ray is also briefly described.—J. F.

**Influence of Irradiation-Killed Cells on Tumor Growth.** Henshaw, P. S. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:305-307. 1943.

The possible influence of irradiation-killed tumor cells on other living tumor cells was studied on mouse sarcoma 37 in young Dilute Brown male mice. With the technic used this tumor produces 100% takes. Previous work has shown that it can be completely inactivated by 5,000 r doses of 200 kv. roentgen rays delivered *in vitro*, and by the same dose applied tangentially to well established tumors *in vivo*. In the present work 3 types of experiments were done. (1) Tumor pulp killed by irradiation *in vitro* was mixed in various proportions with viable tumor pulp, and the mixtures inoculated into mice. (2) Well established small tumors were enveloped in subcutaneously inoculated irradiation-killed pulp. (3) Viable tumor pulp was inoculated in the center of large tumors that had been killed by irradiation *in vivo*. In all cases, the viable cells grew as though no dead cells were present in the immediate neighborhood. The author concludes that, for this tumor, irradiation-killed cells or their decomposition products exert no inhibitory influence on the growth of potentially viable tumor cells.—H. Q. W.

**Experimental Chemotherapy of Tumors in Mice.** Turner, F. C. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:265-269. 1943.

One hundred and thirty-two chemicals, largely organic compounds, were injected repeatedly into mice of several different strains bearing spontaneous or transplanted tumors. No effective therapy was discovered.—R. B.

**Principles of Species Adjustment. I. Continuous Exposure.** Spencer, R. R., and Melroy, M. P. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:249-256. 1943.

Cultures of *Paramecium multimicronucleatum* were exposed continuously to methylcholanthrene (1γ per ml.) for over 4 years. After the 35th culture transfer the carcinogen-exposed paramecia survived longer when placed under partial starvation than did control organisms. After the 50th transfer population levels in the experimental cultures were higher than in the controls. After more than 4 years (140th transfer) the population levels were still higher, but the individual animals were about one-half the size of the control organisms.

Phenanthrene (1γ per ml.) destroyed paramecia after 10 culture transfers.

Paramecium cultures exposed continuously to eosin (10 mgm. per ml.) were first stimulated, then depressed, and finally destroyed after 3 years. The same type of result was obtained with crystal violet (0.4γ per ml.) over a shorter period (35 days).

Cultures of flat worms (*Stenostoma tenuicaudatum*) exposed to phenanthrene or radium irradiation survived for a while but eventually died. Methylcholanthrene produced a temporary increase in the population of worm cultures. The worms were still alive and vigorous after 2 years.—R. B.

**Principles of Species Adjustment. II. Discontinuous Exposure.** Spencer, R. R., Melroy, M. B., and Calnan, D. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:257-263. 1943.

*Escherichia coli* and *Eberthella typhosa* were killed within a few days or weeks by continuous exposure to high temperature (45 or 47° C.) but survived discontinuous exposure (alternate days) apparently indefinitely. During this treatment their heat tolerance was gradually increased.

One series of *Eberthella typhosa* cultures exposed discontinuously to high temperature gave rise to an altered form of organism which grew very rapidly under anaerobic conditions but poorly or not at all under aerobic conditions. It was not agglutinated by *E. typhosa* antiserum, and was non-motile. Smears showed a mixture of gram-positive and gram-negative forms.—R. B.

**The Vertebral Vein System as a Mechanism for the Spread of Metastases.** Batson, O. V. [Graduate Sch. of Med., Univ. of Pennsylvania, Philadelphia, Pa.] *Am. J. Roentgenol.*, **48**:715-718. 1942.

The author believes that our present information regarding the blood-vascular system is inadequate and suggests that more detailed study of it might furnish an explanation of the so-called paradoxes of the spread of metastases and abscesses.

Injection of the dorsal vein of the penis in monkeys, which drains directly into the prostatic plexus of veins, shows, on compression of the inferior vena cava, a direct connection with the vertebral system of veins. This suggests that such a route may be the one by which carcinoma of the prostate spreads to the vertebrae and thence via the inferior vena cava to the general circulation.—J. F.

**Recent Studies in the Production of Cancer by Chemical Compounds; the Conditioned Deficiency as a Mechanism. The Bulkley Lecture.** Rhoads, C. P. [Memorial Hosp., New York, N. Y.] *Bull. New York Acad. Med.*, **18**:53-61. 1942.

A brief review of the most important historical developments in the production of tumors by extrinsic and intrinsic chemical agents, with a presentation of the more recent contributions to the subject.—E. E. S.

# Clinical and Pathological Reports

## Diagnosis—General

**Beitrag zur Frage der klinischen Verwendbarkeit der Abwehrproteinasen-Reaktion nach E. Abderhalden in der Karzinomdiagnostik. [The Abderhalden Reaction in the Diagnosis of Cancer.]** Bauer, J., and Fritze, Th. *Münch. med. Wchnschr.* 1941, 730. Abst. in *Schweiz. med. Wchnschr.*, **71**:1569. 1941.

The authors report on 256 reactions in 52 patients, confirming in general the experience of others.

They believe that the test may be of diagnostic value provided it is repeated at intervals under optimum conditions.—W. H. W.

**Methylene Blue Decolorization Test for Carcinoma.** Barondes, R. de R. [Los Angeles, California] *M. Rec.*, **155**:200-202. 1942.

The author states that in patients with certain cancers there can be crystallized from the urine a substance peculiar to malignant tumors to which the formula $C_7H_8NO_2$ has been given. It is believed to be derived from tryptophane by enzymic decomposition characteristic of cancer. If methylene blue is instilled into the rectum of the patients bearing cancer, the blue urine fades on standing. This also occurs in pregnancy. The author explains various mechanisms that he considers responsible for these phenomena, without substantiating experimental data. (No statistics are given concerning the number of patients on whom these observations have been made nor the type and number of patients used in control studies.) Purely theoretical discussion is included.—E. E. S.

**Tumor Cells in Body Fluids.** Helwig, E. B. [Washington Univ. Sch. of Med., Barnes Hosp. and Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *J. Missouri M. A.*, **39**:73-76. 1942.

The examination of the sediment of fluids from serous cavities seems to be a useful procedure for determining the presence of a neoplasm. The results of such examination of 65 peritoneal and pleural fluids are summarized to indicate the method by which the correct diagnosis ultimately was established. It is concluded that a report of "tumor cells present" should carry with it a high degree of accuracy; a report of "no tumor cells present" is of less diagnostic value. Fragments of tissue composed of stroma and acini or papillae are indicative of malignant cells. Atypical mitotic figures and bizarre multinuclear cells also are indicative. Desquamated cells from the lining of the serous cavities may be confused with tumor cells. Cells arranged in clumps or acini should be accepted as tumor cells only when they exhibit pronounced pleomorphism. Both positive and negative fluids may contain cells in mitotic division, cells with vacuoles, red blood cells, and fibrin.—J. L. M.

## Radiation—Diagnosis and Therapy

**Radiation Osteitis and Fracture Following Irradiation. With a Report of Five Cases of Fractured Clavicle.** Slaughter, D. P. [Memorial Hosp., New York, N. Y.] *Am. J. Roentgenol.*, **48**:201-212. 1942.

The author points out that bone is more susceptible to radiation injury than other tissues because of the increased absorption due to the density of the structure and the increased intensity of secondary radiation. It also has a more vulnerable blood supply because of the inflexible nature of the structure and the fact that in such bones as the mandible, the main blood vessel is enclosed in bone, so that expansion is not possible. The pathological picture resulting from various doses is described, and it is pointed out that smaller doses will produce a reversible reaction while larger ones produce irreversible changes resulting in necrosis and sequestration. The dangers of infection, such as that from an infected tooth, are noted and their effect on mortality figures is remarked. Growing bone is especially susceptible, and radiation over an epiphysis will produce cessation of growth and atrophy of the part. An estimate of the depth dose required to produce these changes is made. In fractures of the femur, clavicle, and ribs, doses varied from 1,500 to 6,000 r, averaging 2,000. The author calls attention to the intense dose delivered to bone from radium placed close to it and advises that shielding of the bones and smaller ports be used in an effort to reduce this complication.—J. F.

**Radiotherapy of Cancer of the Antrum.** Valencia, E., and Rosenthal, L. M. [Chicago Tumor Inst., Chicago, Ill.] *Am. J. Roentgenol.*, **48**:672-676. 1942.

The concentration method of treatment of carcinoma of the antrum is presented. The technic is to deliver increasing doses of X or gamma radiation to gradually diminishing field areas. The method is experimental and is designed to treat lesions that in the past have proved resistant to usual doses. Four cases are presented in only 1 of which is cure attributed to the method described.—J. F.

**Pathologic Aspects of the Radiosensitivity of Malignant Tumors.** Warren, S. [Boston, Mass.] *Am. J. Roentgenol.*, **48**:384-392. 1942.

The author divides malignant tumors into 3 groups, radiosensitive, radioresponsive, and radioresistant, and characterizes each. The fact is noted that it is not the wave length but the amount of energy absorbed that is the important factor in radiation therapy.

The reason for the increased resistance of previously irradiated tissue is discussed, and the explanations offered are the inability of the stroma to respond a second time, and the possibility that the residual cells are those that are less sensitive and had not responded to the original dose.

Detailed study and repeated biopsies during treatment are advised.—J. F.

**Million Volt Roentgen Therapy for Intrathoracic Cancer. Palliative Effects in a Series of Sixty-Three Cases.** Watson, W. L., and Urban, J. [Memorial Hosp., New York, N. Y.] *Am. J. Roentgenol.*, **49**:299-306. 1943.

Sixty-three patients with deep seated intrathoracic cancer were treated at Memorial Hospital during 1940 with the million volt roentgen ray machine. Symptomatic improvement following treatment was shown by 82% of the patients with cancer of the lung for an average duration

of 4 months, and by 55% of those with cancer of the esophagus for an average of 5 months. Previously performed gastrostomies, however, add to the difficulty of estimating the true effect of the roentgen irradiation.—E. H. Q.

**Roentgenologic Examination of the Pharynx.** Westing, S. W. [Brooklyn Cancer Inst., Brooklyn, N. Y.] *Am. J. Roentgenol.*, **49**:587-592. 1943.

The place of roentgenology in the examination of the pharynx is stated briefly. For the lower half of the pharynx and the cervical esophagus a roentgenoscopic technic is outlined that obviates the difficulties ordinarily arising during the opaque meal study of the alimentary tract. Roentgenograms of the normal pharynx are described and reproduced in posteroanterior, lateral, and oblique projections. Three cases of cancer are described: 1 patient was in a very advanced stage of the disease, 1 had definite, but less conspicuous findings, and 1 lacked the customary characteristic history of dysphagia. As cancer frequently affects larynx and hypopharynx simultaneously, the roentgenologist should scrutinize both organs, even when the examination of only one is requested.—E. H. Q.

## Breast

**Cancer of the Breast.** Callaway, E. [La Grange, Ga.] *South. Surgeon*, **11**:394-399. 1942.

A general discussion with emphasis on methods of treatment.—E. E. S.

**Carcinoma of the Breast.** Dulin, J. W. [State Univ. of Iowa, Coll. of Med., Iowa City, Iowa] *J. Iowa M. Soc.*, **32**:172-173. 1942.

A brief general discussion.—J. L. M.

**Carcinoma of the Breast. I—Results of Treatment.** Haagensen, C. D., and Stout, A. P. [Presbyterian Hosp. and Columbia Univ., New York, N. Y.] *Ann. Surg.*, **116**:801-815. 1942.

Data are presented regarding the treatment of 1,040 cases of carcinoma of the breast seen at the Presbyterian Hospital during the years 1915 to 1934 inclusive. Forty-one (6.6%) of 623 ward patients came to the hospital with no symptoms referable to the breast, and the breast tumor was discovered during routine physical examination. Of 640 patients subjected to radical mastectomy, 20 (3.1%) died at operation; within 5 years' time, 41 (6.4%) were lost track of, 13 (2%) died of unknown cause, 12 (1.9%) died of intercurrent disease without evidence of recurrence of carcinoma, 290 (45.3%) died of breast carcinoma, 33 (5.2%) were alive with recurrence, and 231 (36.1%), without recurrence. The incidence of local recurrence was 22.8%. More patients were subjected to longer operative procedures during each successive 5 year period in the study, and as the length of time devoted to the operation increased, the clinical cure rate also rose. There was no evidence that biopsy prejudiced the end results of radical mastectomy. The clinical cure rate (5 years) was 61% among patients without axillary involvement (microscopic) and 21% in those with axillary metastases. Preoperative or postoperative irradiation had little effect on the end results.—W. A. B.

**Extramammary Breast Carcinoma.** Rawls, J. L. [Norfolk, Va.] *Virginia M. Monthly*, **69**:448-449. 1942.

Three cases are reported of carcinoma of the type usually found in breast tissue but appearing in the region of the breast and not within the organ itself. In 2 cases in which a complete operation was done, no evidence of tumor was found in the breast.—M. E. H.

**Intramammary Hemangioma.** Smythe, F. W. [Memphis, Tenn.] *Ann. Surg.*, **115**:716-719. 1942.

A report of 4 cases.—E. A. L.

**Clinical Diagnosis of Cancer of the Breast.** Stout, A. P. [Presbyterian Hosp., New York, N. Y.] *J. Missouri M. A.*, **39**:301-303. 1942.

The technic of breast examination used in the Neoplasm Clinic at the Presbyterian Hospital is described. An outline is presented of the clinical criteria of inoperability, based on the follow-up results of the author's cases of radical mastectomy.—J. L. M.

**Paget's Disease of the Nipple.** West, J. P., and Nickel, W. F., Jr. [New York Hosp. and Cornell Univ. Med. Coll., New York, N. Y.] *Ann. Surg.*, **116**:19-25. 1942.

Twenty cases of Paget's disease of the nipple are reviewed. Two groups are recognized clinically: (1) patients with long standing eczema with or without a tumor in the breast, and (2) those having a recognizable carcinoma of the breast with invasion of the nipple. In the first group prognosis is good with early surgical treatment, but it is poor in the second group. Radical mastectomy is urged for all patients in whom the diagnosis of Paget's disease of the nipple is established.—W. A. B.

**Skin Removal in Radical Mastectomy.** White, W. C. [New York, N. Y.] *Ann. Surg.*, **115**:1182-1185. 1942.

The frequency of local recurrence was studied in a series of 238 cases of carcinoma of the breast and found to be 22.6%. In every case a wide margin of skin had been removed with the breast, and the author concludes that the failures were not due to the method but to the fact that the tumor was already widespread.—E. A. L.

## Female Genital Tract

**Bilateral Ovarian Carcinoma Associated with Intrauterine Pregnancy.** Bossert, L. J. [Coll. of Med. of the Univ. of Cincinnati General Hosp., Cincinnati, Ohio] *Am. J. Obst. & Gynec.*, **44**:336-341. 1942.

Thirteen cases of malignant ovarian disease associated with pregnancy, collected from the literature, are summarized, and a case of bilateral carcinoma of the ovaries occurring in a pregnant woman is described in detail. It is concluded that careful prenatal examination is necessary for the early diagnosis of ovarian tumors in the pregnant woman. Laparotomy is indicated when a proliferating type of ovarian tumor 8 cm. or more in diameter is found. If possible the nature of the tumor should be known at the time of the laparotomy so that whatever procedure is indicated by the exploration can be carried out.—A. K.

**Invasion of the Bony Pelvis by Carcinoma of the Cervix Uteri as a Cause of Pathologic Central Dislocation of the Hip.** Elder, J. R., and Mathieson, N. M.

[Central Middlesex County Hosp., London, England] *Ann. Surg.*, **116**:1-5. 1942.

A report of 4 cases. Carcinoma of the cervix uteri may invade the pelvic wall in the region of the acetabulum as the final stage in a continuous lateral spread. Pain in these patients is often extremely severe probably because of the lesion in the bone and the sacrococcygeal plexus. Massive bone invasion is not frequently seen probably because patients die from sepsis, hemorrhage, or uremia before the bone lesions have had time to develop.—W. A. B.

**Theca Cell Tumor of the Ovary. Report of a Case.** Fallas, R. E. [Los Angeles, Calif.] *West. J. Surg.*, **50**:182-186. 1942.

A case of theca cell tumor of the ovary is presented. There was an opportunity for pathological study twice within 3 years before the tumor was removed and for following the effects of radiation upon this type of ovarian tumor.—M. E. H.

**Primary Carcinoma of Fallopian Tube. Case Report.** Hoenig, E., and Warner, M. P. [New York, N. Y.] *M. Rec.*, **155**:271-273. 1942.

A case in which such a tumor was resected is described. There is a brief summary of important findings in other cases of this disease.—E. E. S.

**Carcinoma of the Cervix. A Study of 233 Cases Including 103 Five-Year Cases with a Survival Rate of 33.9 Per Cent.** Johnston, J. R. [Western Pennsylvania Hosp. Tumor Clinic, Pittsburgh, Pa.] *Pennsylvania M. J.*, **46**: 1062-1067. 1942-43.

In this series of 233 cases, all patients were treated, and treatment was not repeated unless there was a recurrence as proved by biopsy. Late noncarcinomatous postirradiation ulcerations occurred in 4 cases. In the follow-up of these patients only 2 or 0.8% were lost. The results in 103 five-year cases showed a survival rate of 33.9%.—J. L. M.

**Neoplasms of the Ovary.** Meigs, J. V. [Boston, Mass.] *Pennsylvania M. J.*, **46**:797-804. 1942-43.

The author discusses ovarian tumors and draws attention to symptoms, diagnosis, treatment, and special facts about them.—J. L. M.

**The Value of the Vaginal Smear in the Diagnosis of Uterine Cancer.** Meigs, J. V., Graham, R. M., Fremont-Smith, M., Kapnick, I., and Rawson, R. W. [Vincent Memorial Hosp., Pondville Hosp., and Massachusetts General Hosp., Boston, Mass.] *Surg., Gynec. & Obst.*, **77**:449-461. 1943.

The authors studied vaginal smears made by Papanicolaou's method from 220 women in an attempt to make the diagnosis of uterine cancer. The normal cornified cells, and endometrial cells of the vaginal secretions are described, and the criteria are given for the diagnosis of carcinoma. Positive vaginal smear diagnoses were made in 45 out of 46 patients with proved cancer of the cervix and in 11 of 12 with endometrial cancer, while positive smears were also reported in 4 of 153 women presumably free from cancer. It proved possible to make the diagnosis of cancer by vaginal smear examination in 15 early cases. Fifteen black-and-white photomicrographs and 1 plate of colored drawings illustrate the normal and neoplastic cells.—J. G. K.

**Nerve Tumors of the Female Genitals and Pelvis.** Meyer, R. [Univ. of Minnesota, Minneapolis, Minn.] *Arch. Path.*, **36**:437-464. 1943.

A simplified classification of nerve tumors is presented together with a general discussion of the growths of this sort that occur in the female pelvis and genitalia. A number of illustrative cases are cited, and 26 microphotographs are reproduced.—J. G. K.

**Inadequate and Ill-Advised Surgery in the Treatment of Carcinoma of the Cervix.** Scheffey, L. C., and Hahn, G. A. [Jefferson Med. Coll. Hosp., and American Oncologic Hosp., Philadelphia, Pa.] *Pennsylvania, M. J.*, **46**: 1056-1061. 1942-43.

The authors believe that carcinoma of the uterine cervix is best treated by irradiation therapy alone. There may be certain patients, carefully selected, who might possibly have a better chance for longer survival if, following irradiation, they were treated surgically by one experienced in the rigorous technic of the radical operation, but the authors feel that, in general, this is not so. Since a simple type of vaginal hysterectomy or abdominal panhysterectomy is at best only a hazardous and delaying measure in these instances, it is considered best to avoid surgery in the treatment of cervical carcinomas.—J. L. M.

## Male Genital Tract

**Two Years' Personal Experience with Bone Cancer. (A Preliminary Report.)** Brown, O. H. [Arcadia, Calif.] *Urol. & Cutan. Rev.*, **46**:358-361. 1942.

This is a physician's account of his own prostatic carcinoma, which has metastasized widely to bone. A wide variety of therapeutic measures has been tried including the intravenous injection of radioactive strontium. The author believes the latter helped him but that diethylstilbestrol has given "slightly greater" improvement.—V. F. M.

**Report of a Case of Embryonic Carcinoma (Semimona) of the Spermatic Cord.** Cole, A. V., and Campagna, E. A. [East Chicago, Ind.] *Indiana State Medical Ass. J.*, **35**:67-68. 1942.

A boy of 15 had an undescended testis and several small masses lying along the spermatic cord but not involving the testis itself. The Ascheim-Zondek test was negative. Histologic sections of these masses were diagnosed as the title indicates. Cell detail in the photomicrographs reproduced is too poor to enable the reader to form an independent opinion.—E. E. S.

**The Adequate Treatment of Prostatic Disease. With Special Reference to Pathology.** Henline, R. B. [James Buchanan Brady Foundation, New York Hosp., New York, N. Y.] *N. Y. State J. Med.*, **42**:51-53. 1942.

The surgical procedure employed to relieve obstruction in disease of the prostate should not be determined by the surgeon's skill in one method but by the pathological factors involved. Hyperplasia causing obstruction usually occurs in the muscular layer under the urethra, and transurethral removal of the so called adenoma is a rational procedure. In this technic, however, only the hyperplastic tissue is removed, and part or all of the functioning glands of the posterior lobe remain firmly attached to the capsule. To remove the prostate gland proper, a perineal exposure

is necessary. Since malignancy of the prostate is said to originate in the posterior lobe in about 75% of cases, it becomes imperative to consider a radical prostatectomy by the perineal route whenever a malignant growth is suspected.—A. C.

**Effect of Orchiectomy and Irradiation on Cancer of the Prostate.** Huggins, C. [Univ. of Chicago, Chicago, Ill.] *Ann. Surg.*, **115**:1192-1200. 1942.

In a series of 45 patients with advanced prostatic cancer in whom bilateral orchidectomy was the primary treatment, there was no clinical improvement in 5, temporary improvement in 9, and prolonged inhibition in 31 (lasting as long as 30 months). In the more satisfactory cases the cytological picture was adenocarcinoma rather than undifferentiated carcinoma. Estrogen administration was found not to be a useful supplement in the failure cases after orchidectomy. Roentgen irradiation in the doses tested was inadequate as a therapeutic agent.—E. A. L.

**A Summary of Endocrine Effects in Advanced Prostatic Cancer.** Huggins, C. B. [Univ. of Chicago Med. Sch., Chicago, Ill.] *Pennsylvania M. J.*, **46**:1023-1024. 1942-43.

In a series of 47 men with advanced prostatic cancer, it was found that 24 had an elevation of acid and alkaline phosphatases, while in the other 23 the enzymes were within the normal ranges. Decreasing the amount or the activity of the androgens by castration or by administration of estrogen (stilbestrol, 1 mgm. daily) caused a decrease of serum acid phosphatase values, whereas androgen administration (testosterone propionate, 25 mgm. daily) caused an increase of the serum phosphatase values and an exacerbation of the disease.

Of 45 patients treated by castration 14 have died—all with extensive metastases to bone. In 10 of these men, carcinomatosis was the principal cause of death, while in the others it was of secondary importance. From a clinical standpoint, 31 men have had a sustained improvement lasting as long as 30 months; 9 men have had a temporary improvement following castration.—J. L. M.

**Cancer of the Prostate Gland.** Olson, P. F. [Dubuque, Iowa] *J. Iowa M. Soc.*, **32**:169-171. 1942.

Cancer of the prostate gland metastasizes early, often before it becomes diagnosable. Urinary obstruction is a late symptom of the condition, and by the time obstruction occurs the growth has almost always extended beyond the gland. The earlier diagnosis of prostatic cancer cannot be made from the examination of the urologist, who does not see the patient until urinary symptoms develop, but only by attentive rectal palpation of the gland as a part of all physical examinations on men over 50 years of age.

Radical operation offers a prospect of cure in the small percentage of cancers of the prostate gland that can be diagnosed before they cause local symptoms and before they begin to extend through the perineural lymphatics. X-ray therapy alleviates the pain caused by perineural metastases, and transurethral resection offers relief from the distress caused by the obstruction to the outlet of the bladder.—J. L. M.

**Cancer of the Prostate. A Clinico-Pathologic Study of 34 Cases in Negroes.** Quinland, W. S. [Meharry Med. Coll., Nashville, Tenn.] *J. Urol.*, **50**:228-236. 1943.

A review of 34 cases of prostatic cancer in negroes. Cancerous growths varying in size from microscopic inclusions to a mass of 720 gm. were studied. It was found that carcinomatosis was more frequent with the scirrhous type of carcinoma.—V. F. M.

**Metaplasia of the Prostatic Epithelium: A Lesion Sometimes Mistaken for Carcinoma.** Sutton, E. B., and McDonald, J. R. [Mayo Foundation, and Mayo Clinic, Rochester, Minn.] *Am. J. Clin. Path.*, **13**:607-615. 1943.

Fifteen and nine tenths per cent of the 220 prostate glands removed by routine transurethral resection contained islands of metaplastic epithelium. These were especially common in the region of prostatic infarcts and in previously resected glands. Seven figures illustrate the metaplastic change, which is strictly benign and without clinical significance except when misinterpreted as carcinoma.—J. G. K.

**Carcinoma of the Prostate.** Wilhelmi, O. J. [St. Louis, Mo.] *J. Urol.*, **50**:341-343. 1943.

The author believes urinary acid phosphatase will be a more accurate indication of prostatic cancer activity than the blood acid phosphatase. Orchidectomy is advised in early carcinoma. Recurrence of symptoms after orchidectomy was present in one-third of the author's cases within the first year.—V. F. M.

## Urinary System—Male and Female

**Total Cystectomy with Bilateral Nephrostomy for Carcinoma of the Bladder. Five-Year Result.** Hyman, A. [New York, N. Y.] *Ann. Surg.*, **114**:149-150. 1941.

Report of a case 5 years after operation. In a series of 27 cystectomies the operative mortality was 22%. Fifty per cent of the patients surviving operation have lived for 5 years or longer.—M. R. D.

**Fatty Substitution of the Kidney or Lipomatosis Renalis.** Nolan, L. E., and Engelfried, C. H. [Laird Mem. Hosp., Montgomery, W. Va.] *Urol. & Cutan. Rev.*, **46**:624-626. 1942.

Fatty substitution is due to hyperplasia of adipose tissue replacing atrophic parenchyma. The atrophy may be caused by any of the usual diseases destroying renal tissue. This condition is therefore not neoplastic, and is distinct from renal lipoma, retroperitoneal lipoma, or sarcoma.—V. F. M.

**Papillary Carcinoma of the Ureter.** Reagan, J. R. [Wichita Falls, Tex.] *J. Urol.*, **50**:304-306. 1943.

The author reports a case of papillary carcinoma of the ureter in a man of 33 and adds a brief discussion.—V. F. M.

**Papillary Carcinoma of the Kidney in Childhood. (Report of a Case with a Ten-Year Cure.)** Schlapik, D. [Chicago, Ill.] *Urol. & Cutan. Rev.*, **47**:283-286. 1943.

A female, aged 13 years, had a nephrectomy for a walnut sized papillary tumor of the renal pelvis. Histologically, the diagnosis of papillocarcinoma was made. The patient is in good condition 10 years later.—V. F. M.

**Intraperitoneal Rupture of a Wilms' Tumour.** Tanner, C. H. [London Hosp., London, England] *Brit. M. J.*, **2**:714. 1943.

Description of a case.—E. L. K.

**Caruncle of the Urethra in the Female with Special Reference to the Importance of Histological Examination in the Differential Diagnosis.** WALTHER, H. W. E. [Southern Baptist Hosp., New Orleans, La.] *J. Urol.*, **50**:380-388. 1943.

The author studied 2 groups of patients with lesions of the female external urinary meatus. In the first series 4 of 15 specimens taken for histologic examination were diagnosed as carcinoma. In the second series 1 of 32 specimens showed malignant change. Clinically, benign and malignant lesions cannot with certainty be differentiated unless the carcinoma is advanced, hence biopsy is essential. Approximately 275 instances of carcinoma of the female urethra are reported in the literature.—V. F. M.

## GASTROINTESTINAL TRACT

**Duodenal Tumor of Unusual Character.** SHACKELFORD, R. T., FISHER, A. M., and FIROR, W. B. [Baltimore, Md.] *Ann. Surg.*, **116**:864-873. 1942.

This is a report of a myosarcoma of the duodenum in a patient who had had a myosarcoma of the uterus removed 4 years previously. The patient had a sprue-like diarrhea associated with anemia and blood in the stools. X-ray studies suggested a benign rather than a malignant tumor. Resection of the lesion was successfully performed, but the patient succumbed probably to metastases about 9 months after operation.—W. A. B.

**Myxofibroma of the Jejunum.** SULLIVAN, T. F. X., and CORCORAN, W. L. [Saint Clare's Hosp., New York, N. Y.] *New York State J. Med.*, **42**:149-150. 1942.

Myxofibroma is extremely rare, particularly in the jejunum. A case of this type is reported.—J. L. M.

**Cancer of the Cervical Esophagus.** WATSON, W. L. [Memorial Hosp., New York, N. Y.] *Ann. Surg.*, **116**:86-97. 1942.

One patient is reported as living and well 5½ years after combined radon seed implantation and external x-ray irradiation for epidermoid carcinoma of the cervical esophagus with metastases to cervical lymph nodes. A second patient with a similar condition is free from evident tumor 4 years after irradiation. A method for the surgical treatment of carcinoma of the cervical esophagus is described. One patient has survived the procedure for 18 months.—W. A. B.

**Retention of the Sphincter in the Radical Operation for Carcinoma of the Rectum and Rectosigmoid.** WILENSKY, A. O. [New York, N .Y.] *New York State M. J.*, **42**:1150-1154. 1942.

This paper serves to emphasize resection of the rectum or rectosigmoid in continuity with retention of the sphincter, regardless of the special type and method of approach. Many of the standard forms of operation include a permanent colostomy. To many patients this is not voluntarily acceptable if there is any other choice; they accept it as imperative in the fear of contemplated consequences. It is the conviction of the author that many times there is a good deal of unnecessary ruthlessness in the sacrifice of the sphincter. The following topics are discussed in support of this belief: (1) anatomic considerations, (2) histologic data, (3) clinical considerations, (4) biopsies, (5) technical considerations such as radical operability, conservation of the sphincter, resection of the rectum in continuity, methods of approach, posterior operations, combined operations, multiple stage operations, and "drawing through" operation, and (6) the case reports of 8 patients.—J. L. M.

**Adenoacanthoma of the Pyloric End of the Stomach. A Consideration of Its Histogenesis and a Report of Two Cases.** WOOD, D. A. [Stanford Univ., Sch. of Med., San Francisco, Calif.] *Arch. Path.*, **36**:177-189. 1943.

The tumors are believed to arise from undifferentiated basal cells in the gastric mucosa.—J. G. K.

## LIVER

**Solitary Nonparasitic Cyst of the Liver.** MUNROE, H. S. [Charlotte, N. C.] *Ann. Surg.*, **116**:751-762. 1942.

A report of a case in a 9 month old infant. Marsupialization was done with a satisfactory result.—W. A. B.

**Common Duct Obstruction Due to Primary Carcinoma of the Cystic Duct. Resection with Reestablishment of Continuity of the Common Bile Duct.** OPPENHEIMER, G. D. [Mt. Sinai Hosp., New York, N. Y.] *Ann. Surg.*, **116**:141-146. 1942.

A report of a case. Continuity of the common bile duct was reestablished by means of a rubber T tube.—W. A. B.

**Malignant Hepatoma.** ROBILLARD, G. L., and GOLDMAN, C. [Norwegian Hosp., and Cumberland Hosp., Brooklyn, N. Y.] *Am. J. Surg.*, **61**:430-433. 1943.

Case report.—W. A. B.

## BONE AND BONE MARROW

**Chondro-Sarcoma of the Rib. Five Year Cure after Resection.** MAYER, L. [Hosp. for Joint Diseases, New York, N. Y.] *J. Mt. Sinai Hosp.*, **7**:467-470. 1941.

Although chondromas are considered to be benign lesions, they display a decided tendency to recur locally after surgical removal. The recurrence is often sarcomatous, and for this reason wide excision of a recurrent chondroma is indicated. The patient described is well 5 years after the removal of a recurrent osteochondroma of the rib that involved the entire chest wall and showed sarcomatous degeneration.—A. Cnl.

**Osteochondroma of the Coronoid Process of the Mandible.** SHACKELFORD, R. T., and BROWN, W. H. [Johns Hopkins Hosp., Baltimore, Md.] *Surg., Gynec. & Obst.*, **77**: 51-54. 1943.

Two cases with 7 figures are presented and discussed.—I. G. K.

## PANCREAS

**Cystadenoma of the Pancreas. Report of Five Cases.** BOWERS, R. F., LORD, J. W., JR., and MCSWAIN, B. [New York Hosp. and Cornell Univ. Med. Coll., New York, N. Y.] *Arch. Surg.*, **45**:111-122. 1942.

Report of 5 cases.—G. H. H.

**The Surgery of Carcinoma of the Pancreas and Ampullary Region. Report of Six Additional Cases.** HARVEY, S. C., and OUGHTERSON, A. W. [Yale Univ. Sch. of Med., New Haven, Conn.] *Ann. Surg.*, **115**:1066-1090. 1942.

The cases are discussed with particular emphasis on early signs and symptoms, laboratory data with reference

to disturbances in fat and carbohydrate metabolism, and surgical management. Contrary to the usual belief, upper abdominal pain is one of the most common presenting complaints. Others are jaundice, profound and rapid weight loss, and nausea and vomiting. Hyperglycemia is found in 20% of all patients. Disturbed fat metabolism is not common. The evidence is increasing that a one stage resection is preferable to a multiple stage procedure.—E. A. L.

**Cysts of the Pancreas.** Rabinovitch, J., and Pines, B. [Jewish Hosp., Brooklyn, N. Y.] *Arch. Surg.*, **45**:727-746. 1942.

A detailed report of 17 instances of cysts of the pancreas. The lesions included 11 pseudocysts, 2 cystadenomas, 2 retention cysts, 1 inclusion cyst of Brunner's gland, and 1 cyst with carcinomatous change.—G. H. H.

**A Consideration of the Surgical Treatment of Tumors of the Pancreas.** Whipple, A. O. [Columbia Univ., New York, N. Y.] *J. Tennessee M. A.*, **35**:179-189. 1942.

A brief résumé of some important discoveries concerning the pancreatic functions is given. Seale Harris is credited with the origin of the idea that pancreatic islet tumor might cause insulin shock. W. J. Mayo removed an islet carcinoma in 1927, and since then about 100 cases of tumor arising in the islands have been reported. The great majority of these are benign. They initiate the syndrome of central nervous system disorder coming on during fasting, low fasting blood sugar levels, and relief by glucose administration. Hepatic, pituitary, adrenal, and thyroid abnormality should be ruled out, and surgical exploration carried out promptly. The details of operation are given. The size of the tumor bears no relation to the severity of symptoms. Occasionally thyroid storm has followed removal of a pancreatic tumor. The remainder of the article is devoted to the technic and rationale of radical pancreatectomy for malignant pancreatic tumors.—E. E. S.

## Miscellaneous

**Retroperitoneal Tumors.** Acuff, H. [Knoxville, Tenn.] *South. Surgeon*, **11**:200-209. 1942.

The types of cystic and solid tumors occurring primarily in this region are listed and discussed, and several illustrative cases are presented.—E. E. S.

**An Intrapericardial Teratoma and a Tumor of the Heart: Both Removed Operatively.** Beck, C. S. [Western Reserve Univ. and University Hosps., Cleveland, Ohio] *Ann. Surg.*, **116**:167-174. 1942.

In the cases reported both patients survived.—W. A. B.

**Malignant Teratoma of the Arm. Case Report.** Christy, C. J. [State Inst. for the Study of Malignant Disease, Buffalo, N. Y.] *Ann. Surg.*, **116**:948-949. 1942.

A case report.—W. A. B.

**A Pedunculated Lipoma.** Johnson, J. A. W. [Newton, Iowa] *J. Iowa M. Soc.*, **32**:71-72. 1942.

A case is presented of a patient with toxic adenoma of the thyroid gland, lipoma below the distal half of the left clavicle, and pedunculated lipoma of the upper anteromedian aspect of the left thigh.—J. L. M.

**Sacrococcygeal Teratoid Tumor. Case Report.** Pack, G. T., and Braund, R. [Memorial Hosp., New York, N. Y.] *Ann. Surg.*, **116**:76-80. 1942.

A report concerning a year old infant from whom the tumor was successfully removed.—W. A. B.

**Clinical and Pathological Aspects of Certain "Hormone-Producing" Tumors.** Reimann, S. P., and Keller, E. B., Jr. [Lankenau Hosp. Research Inst., Philadelphia, Pa.] *Pennsylvania M. J.*, **46**:805-808. 1942-43.

It is possible to diagnose the presence of certain "hormone-producing" tumors from clinical symptoms. Among the neoplasms presented are granulosa-cell tumors, arrhenoblastomas, and theca-cell tumors of the ovary, various adrenal tumors, testicular, pituitary, and thyroid tumors.—J. L. M.

**Long-Period Recurrences in Cancer.** Webster, J. H. D. [Meyerstein Inst. of Radiotherapy, Middlesex Hosp., London, England] *Brit. M. J.*, **2**:720. 1943.

"Almost all recurrences of cancer appear at intervals of a few months or years, and recent studies have shown that when the onsets of active phases of growth can be dated to a few weeks they may be timed at intervals which are multiples of 7.6 months (33 weeks $\pm$ 3 weeks) or of the half-period."—E. L. K.

## Cancer Control and Public Health

**Cancer Prevention Clinics.** Editorial. *Rhode Island M. J.*, **26**:300-301. 1943.

Approval has been given by the Rhode Island Medical Society for the establishment of "Cancer Prevention Clinics" in that state.—M. E. H.

**Operation of the Tumor Clinic at Hines, Ill., During the Calendar Year 1941.** Schrek, R. [Veterans Admin., Hines, Ill.] *M. Bull. Vet. Admin.*, **20**:181-186. 1943.

This report is chiefly statistical but contains some comments relative to racial and geographical distribution of patients and the types of treatment given. An analysis of 1,770 primary admissions and 731 readmissions for the calendar year 1941 revealed the most frequent types of tumor seen in these patients to be carcinoma of exposed skin, lower lip, stomach, rectum, and bladder. Tumors relatively more common in white than in negro males were carcinoma of the exposed skin, lower lip, and tongue, and tumors of the brain and meninges. More frequent in negroes were carcinoma of the prostate, penis, scrotum, tonsil, pharynx, nasopharynx, esophagus, and stomach. The Southern states contributed a relatively large number of patients with carcinoma of the exposed skin and lower lip, and a relatively low percentage of patients with carcinoma of the bladder and kidney.—M. E. H.